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I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR 1381 for a patent by AGRICULTURE VICTORIA SERVICES PTY. LTD. , PIG-RESEARCH AND DEVELOPMENT CORPORATION and PFIZER PRODUCTS, INC. filed on 10 November 2000.

I further certify that the above application is now proceeding in the name of AGRICULTURE VICTORIA SERVICES PTY. LTD. , AUSTRALIAN PORK LIMITED and PFIZER PRODUCTS, INC. pursuant to the provisions of Section 113 of the Patents Act 1990.

WITNESS my hand this  
Twenty-sixth day of November 2001

A handwritten signature in cursive script that reads "J R Yabsley".

JONNE YABSLEY  
TEAM LEADER EXAMINATION  
SUPPORT AND SALES

--- OF BLANK (USPTO)



Agriculture Victoria Services Pty. Ltd.  
Australian Pork Limited AND  
~~Pig Research and Development Corporation~~ AND  
Pfizer Products, Inc.

**A U S T R A L I A**  
**Patents Act 1990**

**PROVISIONAL SPECIFICATION**

for the invention entitled:

"Novel therapeutic compositions for treating infection by *Lawsonia spp.*"

The invention is described in the following statement:

## Novel therapeutic compositions for treating infection by *Lawsonia spp.*

### FIELD OF THE INVENTION

The present invention relates generally to therapeutic compositions for the treatment  
5 and/or prophylaxis of intestinal disease conditions in animals and birds caused or  
exacerbated by *Lawsonia intracellularis* or similar or otherwise related microorganism.

In particular, the present invention provides a novel gene derived from *L. intracellularis*  
which encodes an immunogenic polypeptide. The polypeptide described herein,  
selected from the group consisting of flhB, flhR, ntrC, glhH, motA, motB, tlyC, ytfM, and  
10 ytfN polypeptides, or a homologue, analogue or derivative of any one or more of said  
polypeptides, is particularly useful as an antigen in vaccine preparation for conferring  
humoral immunity against *L. intracellularis* and related pathogens in animal hosts. The  
present invention is also directed to methods for the treatment and/or prophylaxis of  
such intestinal disease conditions and to diagnostic agents and procedures for  
15 detecting *L. intracellularis* or similar or otherwise related microorganisms.

### GENERAL

Bibliographic details of the publications numerically referred to in this specification are  
collected at the end of the description. All patents, patent applications, and  
20 publications cited herein are incorporated by reference in their entirety.

Reference hereinafter to "*Lawsonia intracellularis*" or its abbreviation "*L. intracellularis*"  
includes all microorganisms similar to or otherwise related to this microorganism, as  
described by Stills (1991) or Jones *et al.* (1997) or Lawson *et al.* (1993) or McOrist *et*  
25 *al.* (1995).

References herein to "AGAL" shall be taken to mean a reference to the Australian  
Government Analytical Laboratories located at 1 Suakin Street, Pymble, New South  
Wales 2073, Australia. All biological deposits referred to herein in respect of the  
30 plasmids assigned AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glhH);  
NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR);

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NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM) have been made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

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As used herein, the word "*flhB*", or the term "*flhB* gene", shall be taken to refer to a gene encoding the antigenic *flhB* polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 1 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#2 which has been deposited under AGAL Accession No. NM00/16477. The word "*flhB*" or the term "*flhB* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 1 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#2 which has been deposited under AGAL Accession No. NM00/16477. It shall also be understood that the term "*flhB* polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 2 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#2 which has been deposited under AGAL Accession No. NM00/16477. The term "*flhB* polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 2 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#2 which has been deposited under AGAL Accession No. NM00/16477.

As used herein, the word "*fliR*", or the term "*fliR* gene", shall be taken to refer to a gene encoding the antigenic *fliR* polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 3 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#3 which has been deposited under AGAL Accession No. NM00/16478. The word "*fliR*" or the term "*fliR* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 3, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#3 which has been deposited under AGAL Accession

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No.NM00/16478. It shall also be understood that the term "fliR polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 4 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#3 which has been deposited under AGAL Accession No.NM00/16478.

5 The term "fliR polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 4 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#3 which has been deposited under AGAL Accession No.NM00/16478.

10 As used herein, the word "*ntrC*", or the term "*ntrC* gene", shall be taken to refer to a gene encoding the antigenic *ntrC* polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 5 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#6 which has been deposited under AGAL Accession No.NM00/16481. The word "*ntrC*" or the term  
15 "*ntrC* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 5, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#6 which has been deposited under AGAL Accession No.NM00/16481. It shall also be understood that the term "*ntrC* polypeptide" refers to a polypeptide of the invention which comprises the amino acid  
20 sequence set forth in SEQ ID NO: 6 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#6 which has been deposited under AGAL Accession No.NM00/16481. The term "*ntrC* polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 6 or a polypeptide encoded by the *L. intracellularis*  
25 gene contained in the plasmid pGTE#6 which has been deposited under AGAL Accession No.NM00/16481.

As used herein, the word "*glnH*", or the term "*glnH* gene", shall be taken to refer to a gene encoding the antigenic *glnH* polypeptide of the present invention, which gene  
30 comprises the nucleotide sequence set forth in SEQ ID NO: 7 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#1 which has

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been deposited under AGAL Accession No.NM00/16476. The word "*glnH*" or the term "*glnH* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 7, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#1 which has been deposited under

5 AGAL Accession No.NM00/16476. It shall also be understood that the term "glnH polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 8 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#1 which has been deposited under AGAL Accession No.NM00/16476. The term "glnH polypeptide" shall further be taken to

10 include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 8 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#1 which has been deposited under AGAL Accession No.NM00/16476.

15 As used herein, the word "*motA*", or the term "*motA* gene", shall be taken to refer to a gene encoding the antigenic *motA* polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 9, or to the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#4 which has been deposited under AGAL Accession No.NM00/16479 and which has homology to

20 SEQ ID NO: 9. The word "*motA*" or the term "*motA* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 9, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#4 which has been deposited under AGAL Accession No.NM00/16479 and which has homology to SEQ ID NO: 9. It shall also be understood that the term "*motA*

25 polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 10 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#4 which has been deposited under AGAL Accession No.NM00/16479 and which has homology to SEQ ID NO: 9. The term "motA polypeptide" shall further be taken to include a polypeptide which is functionally-

30 related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 10 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#4

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which has been deposited under AGAL Accession No.NM00/16479 and having homology to SEQ ID NO: 9.

As used herein, the word "*motB*", or the term "*motB* gene", shall be taken to refer to  
5 a gene encoding the antigenic *motB* polypeptide of the present invention, which gene  
comprises the nucleotide sequence set forth in SEQ ID NO: 11 or the nucleotide  
sequence of the *L. intracellularis* gene contained in the plasmid pGTE#4 which has  
been deposited under AGAL Accession No.NM00/16479 and having homology to SEQ  
ID NO: 11. The word "*motB*" or the term "*motB* gene" shall further be taken to include  
10 a degenerate or complementary nucleotide sequence to SEQ ID NO: 11, or the  
nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#4  
which has been deposited under AGAL Accession No.NM00/16479 and having  
homology to SEQ ID NO: 11. It shall also be understood that the term "*motB*  
polypeptide" refers to a polypeptide of the invention which comprises the amino acid  
15 sequence set forth in SEQ ID NO: 12 or a polypeptide encoded by the *L. intracellularis*  
gene contained in the plasmid pGTE#4 which has been deposited under AGAL  
Accession No.NM00/16479 and having homology to SEQ ID NO: 11. The term "*motB*  
polypeptide" shall further be taken to include a polypeptide which is functionally-related  
to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 12 or a  
20 polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#4  
which has been deposited under AGAL Accession No.NM00/16479 and having  
homology to SEQ ID NO: 11.

As used herein, the word "*tlyC*", or the term "*tlyC* gene", shall be taken to refer to a  
25 gene encoding the antigenic *tlyC* polypeptide of the present invention, which gene  
comprises the nucleotide sequence set forth in SEQ ID NO: 13 or the nucleotide  
sequence of the *L. intracellularis* gene contained in the plasmid pGTE#5 which has  
been deposited under AGAL Accession No.NM00/16480. The word "*tlyC*" or the term  
"*tlyC* gene" shall further be taken to include a degenerate or complementary nucleotide  
30 sequence to SEQ ID NO: 13, or the nucleotide sequence of the *L. intracellularis* gene  
contained in the plasmid pGTE#5 which has been deposited under AGAL Accession

No.NM00/16480. It shall also be understood that the term "tlyC polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 14 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#5 which has been deposited under AGAL Accession No.NM00/16480.

5 The term "tlyC polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 14 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#5 which has been deposited under AGAL Accession No.NM00/16480.

10 As used herein, the word "ytfM", or the term "ytfM gene", shall be taken to refer to a gene encoding the antigenic ytfM polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 15 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#7 which has been deposited under AGAL Accession No.NM00/16482. The word "ytfM" or the term  
15 "ytfM gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 15, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#7 which has been deposited under AGAL Accession No.NM00/16482. It shall also be understood that the term "ytfM polypeptide" refers to a polypeptide of the invention which comprises the amino acid  
20 sequence set forth in SEQ ID NO: 16 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#7 which has been deposited under AGAL Accession No.NM00/16482.

The term "ytfM polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ  
25 ID NO: 16 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#7 which has been deposited under AGAL Accession No.NM00/16482.

As used herein, the word "ytfN", or the term "ytfN gene", shall be taken to refer to a gene encoding the antigenic ytfN polypeptide of the present invention, which gene  
30 comprises the nucleotide sequence set forth in SEQ ID NO: 17. The word "ytfN" or the term "ytfN gene" shall further be taken to include a degenerate or complementary

nucleotide sequence to SEQ ID NO: 17. It shall also be understood that the term "ytfN polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 18. The term "ytfN polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-  
5 reactive with the polypeptide of SEQ ID NO: 18.

As used herein the words "from" or "of", and the term "derived from" shall be taken to indicate that a specified product, in particular a macromolecule such as a polypeptide, protein, gene or nucleic acid molecule, antibody molecule, Ig fraction, or other  
10 macromolecule, or a biological sample comprising said macromolecule, may be obtained from a particular source, organism, tissue, organ or cell, albeit not necessarily directly from that source, organism, tissue, organ or cell.

Throughout this specification, unless the context requires otherwise, the word  
15 "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

20 Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all  
25 combinations or any two or more of said steps, features, compositions and compounds.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purposes of exemplification only.  
30 Functionally equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

## BACKGROUND OF THE INVENTION

The meat-producing sector of the agricultural industry is dependent upon the health of its livestock and there is a need to maintain disease-free livestock for human consumption. The industry is subject to rapid economic downturn in response to disease conditions adversely affecting livestock and the quality of meat products derived therefrom, including those diseases which may potentially be transmitted to humans. It is important, therefore, to have well defined treatments and prophylactic and diagnostic procedures available to deal with infections or potential infections in livestock animals and humans.

Meat products derived from porcine and avian species are significant commercial products in the agriculture industry. In particular, pigs form a major component of the meat industry. However, pigs are sensitive to a wide spectrum of intestinal diseases collectively referred to as porcine proliferative enteropathy (PPE). These diseases have previously been known as intestinal adenomatosis complex (Barker and van Drumel, 1985), porcine intestinal adenomatosis (PIA), necrotic enteritis (Rowland and Lawson, 1976), proliferative haemorrhagic enteropathy (Love and Love, 1977), regional ileitis (Jonsson and Martinsson, 1976), haemorrhagic bowel syndrome (O'Neil, 1970), porcine proliferative enteritis and *Campylobacter* spp – induced enteritis (Straw, 1990).

There are two main forms of PPE: a non-haemorrhagic form represented by intestinal adenomatosis which frequently causes growth retardation and mild diarrhoea; and a haemorrhagic form, which is often fatal, represented by proliferative haemorrhagic enteropathy (PHE), where the distal small intestine lumen becomes engorged with blood. PPE has been reported in a number of animal species including pigs (McOrist *et al*, 1993), hamsters (Stills, 1991), ferrets (Fox *et al*, 1989), guinea pigs (Elwell *et al*, 1981), rabbits (Schodeb and Fox, 1990) as well as avian species (Mason *et al*, 1998).

PPE is a significant cost component associated with the pig industry, especially in terms of stock losses, medication costs, reduced growth rates of pigs and increased

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feed costs. PPE also contributes to downstream indirect costs in, for example, additional labour costs and environmental costs in dealing with antibiotic residue contamination, and in control measures to prevent the organism from being passed on or carried to other animals or humans.

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*L. intracellularis* is a causative agent of PPE (McOrist *et al.*, 1995). *L. intracellularis* is an intracellular, possibly obligate intracellular, bacterium. It can only be cultured *in vitro* with tissue culture cells (Jones *et al.*, 1997; Lawson *et al.*, 1993; McOrist *et al.*, 1995; International Patent Application No. PCT/US96/09576). *L. intracellularis* is

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located in the cytoplasm of the villus cells and intestinal crypt cells of infected animals. Pigs suffering from PPE are characterised by irregularities in the villus cells and intestinal crypt structure with epithelial cell dysplasia, wherein crypt abscesses form as the villi and intestinal crypts become branched and fill with inflammatory cells.

15

Current control strategies for PPE rely on the use of antibacterials. However, such a strategy is considered to only be short to medium term, especially since governmental regulatory pressures tend to discourage animal husbandry practices which involve the use of prophylactic antibiotics. There is a need, therefore, to develop effective, safe and low cost alternatives to the use of antibiotics and, in particular, to develop vaccine preparations capable of conferring protective immunity against *L. intracellularis* infection in livestock animals.

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The most effective vaccine preparations are generally comprised of a highly antigenic component, such as a polypeptide or other macromolecule which is derived from the pathogenic organism against which the vaccine is directed, wherein said antigenic component produces little or no contraindications when administered to a susceptible host animal, and produces little or no antigenic cross-reactivity with desirable organisms, such as non-pathogenic organisms that are a part of the normal flora of the intestinal tract or other tissues of said host animal. In summary, an effective vaccine preparation must be immunogenic, specific and safe.

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Accordingly, there is a need to identify highly immunogenic antigens produced by the bacterium *L. intracellularis*.

International Patent Application No. PCT/AU96/00767 describes several *L. intracellularis* partial genetic sequences, and partial polypeptides encoded thereby. However, there is a need to further identify polypeptide immunogens produced by the bacterium *L. intracellularis* and immunogenic peptides derived therefrom, including those immunogens which are genus- or species-specific, for use in improved vaccine compositions. The presently-described invention provides such immunogens.

#### SUMMARY OF THE INVENTION

One aspect of the present invention is directed to an isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of a polypeptide derived from *Lawsonia spp*, in particular a polypeptide selected from the group consisting of flhB, flhR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue or derivative of any one or more of said polypeptides.

Preferably, the isolated or recombinant immunogenic polypeptide is selected from the group consisting of the following:

- (i) a polypeptide which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;
- (ii) a polypeptide which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glhH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

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(iii) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(iv) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(v) a homologue, analogue or derivative of any one of (i) to (iv) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

In an alternative preferred embodiment, the isolated or recombinant immunogenic polypeptide is selected from the group consisting of the following:

(i) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(ii) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to the nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a polypeptide encoded by at least about 15 contiguous nucleotides of a

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nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(v) a homologue, analogue or derivative of any one of (i) to (iv) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

10 In a particularly preferred embodiment, the polypeptide of the present invention comprises or consists of an amino acid sequence selected from the group consisting of:

(i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18; and

15 (ii) an amino acid sequence encoded by *L. intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

A further aspect of the present invention provides a vaccine composition for the prophylaxis or treatment of infection in an animal, such as a pig or bird, by *L. intracellularis* or a similar or otherwise related microorganism, said vaccine composition comprising an immunologically effective amount of an immunogenic component which comprises an isolated or recombinant polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides as described herein and one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

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A further aspect of the invention extends to an immunologically interactive molecule,

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such as an antibody or antibody fragment, which is capable of binding to an immunogenic polypeptide of the invention selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides.

- 5 A further aspect of the invention provides a method of diagnosing infection of an animal by *L. intracellularis* or a related microorganism, said method comprising the steps of contacting a biological sample derived from said animal with an immunologically interactive molecule of the present invention for a time and under conditions sufficient for a complex, such as an antigen:antibody complex, to form, and  
10 then detecting said complex formation.

- A further aspect of the present invention contemplates a method of determining whether or not an animal has suffered from a past infection, or is currently infected, by *L. intracellularis* or a related microorganism, said method comprising contacting a  
15 tissue or fluid sample, such as blood or serum derived from said animal, with an immunogenic polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a peptide derived therefrom, for a time and under conditions sufficient for a complex, such as an antigen:antibody complex, to form, and then detecting said complex formation.

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- A further aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides that encodes, or is complementary to a nucleic acid molecule that encodes, a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, including any  
25 and all genes selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes as defined hereinabove.

- In a preferred embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide that is immunologically cross-reactive with *L.*  
30 *intracellularis* or other causative agent of PPE, wherein said nucleotide sequence is selected from the group consisting of:

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(i) a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(ii) a nucleotide sequence having at least about 60% sequence identity overall to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) a nucleotide sequence which hybridizes under at least low stringency, more preferably moderate stringency, and most preferably high stringency conditions to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17 or a complementary nucleotide sequence thereto;

(vi) a nucleotide sequence which hybridizes under at least low stringency, more preferably moderate stringency, and most preferably high stringency conditions to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5

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tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(vii) a homologue, analogue or derivative of any one of (i) to (vi) which encodes a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

5

In a particularly preferred embodiment, the isolated nucleic acid molecule of the present invention comprises or consists of a nucleotide sequence selected from the group consisting of:

- 10 (i) a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1,3, 5, 7, 9, 11, 13, 15, and 17;
- (ii) a nucleotide sequence of the *L. intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glhH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); 15 NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and
- (iii) a nucleotide sequence that encodes the same polypeptide as (i) or (ii), wherein said polypeptide is selected from the group consisting of flhB, fliR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN;
- 20 (iv) a nucleotide sequence that is complementary to (i) or (ii) or (iii); and
- (v) a nucleotide sequence that hybridises under high stringency conditions to the complement of a sequence selected from the group consisting of: SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15 and 17, wherein said nucleotide sequence is the complement of a sequence that encodes a polypeptide that is 25 immunologically cross-reactive to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN.

A still further aspect of the invention provides a diagnostic method of detecting *L. intracellularis* or related microorganism in a biological sample derived from an animal 30 subject, said method comprising the steps of hybridising one or more polynucleotide or oligonucleotide probes or primers derived from a gene selected from the group

consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes, or a homologue, analogue or derivative thereof, to said sample, and then detecting said hybridisation using a detection means. The detection means according to this aspect of the invention is any nucleic acid-based hybridisation or amplification reaction.

5

A further aspect of the invention provides an isolated probe or primer derived from a gene selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes. In a particularly preferred embodiment, the probe or primer of the invention is useful for isolating the *ytfM* and/or *ytfN* genes described herein. More preferably, the probe or primer of the invention comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 19 to SEQ ID NO: 46 or a complementary nucleotide sequence thereto.

10

#### DETAILED DESCRIPTION OF THE INVENTION

In work leading up to the present invention, the inventors sought to identify immunogenic proteins of *L. intracellularis* for use in vaccines for the prophylaxis and treatment of PPE in animals, including pigs and birds.

15

Accordingly, one aspect of the present invention is directed to an isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of a polypeptide derived from *Lawsonia spp.* selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN*, or a homologue, analogue or derivative of any one or more of said polypeptides.

20

Epitopes of *Lawsonia spp.* may be B cell epitopes or T-cell epitopes. It is well-known that antibody-binding sites (B-cell epitopes) involve linear as well as conformational epitopes (van Regenmortel, 1992). B-cell epitopes are predominantly conformational. In contrast, T-cells recognize predominantly linear epitope sequences in combination with MHC class II molecules.

25

30

A precise identification and careful selection of epitopes of *Lawsonia spp.* facilitates

the development of diagnostic reagents and vaccine compositions for the effective treatment or prophylaxis of *Lawsonia* infections. Epitope identification and characterization (i.e., determination of the molecular weight, amino acid sequence, and structure of epitopes of *Lawsonia spp.*) may be performed using art-recognised techniques. For the detection of conformational epitopes, degrading and denaturing of the epitope molecule must be avoided in order to conserve the three-dimensional structure, because the antigen-antibody reaction will be diminished if the secondary structure of the epitope is altered significantly. In practice, the characterisation and isolation of linear non-conformational epitopes is easier, because any immunoreactive regions are contained within a single polypeptide or peptide fragment which is capable of being purified under a range of conditions.

Both non-conformational and conformational epitopes may be identified by virtue of their ability to bind detectable amounts of antibodies (such as IgM or IgG) from sera of animals immunised against or infected with *Lawsonia spp.* and, in particular *L. intracellularis*, or an isolated polypeptide derived therefrom or, alternatively, by virtue of their ability to bind detectable amounts of antibodies in a purified Ig fraction derived from such sera. The antibodies may be derived from or contained within pools of polyclonal sera, or may be monoclonal antibodies. Antibody fragments or recombinant antibodies, such as those expressed on the surface of a bacteriophage or virus particle, such as in a phage display library, may also be employed.

The determination of T-cell epitopes is performed by analysing the ability of the epitope peptides to induce the proliferation of peripheral blood lymphocytes or T-cell clones. The identification of T-cell epitopes is accomplished using a variety of methods as known in the art, including the use of whole and fragmented native or recombinant antigenic protein, as well as the more commonly employed "overlapping peptide" method. In the latter method, overlapping peptides which span the entire sequence of a polypeptide derived from *Lawsonia spp.* are synthesized and tested for their capacity to stimulate T-cell cytotoxic or proliferative responses *in vitro*.

Structure determination of both conformational non-linear and non-conformational linear epitopes may be performed by nuclear magnetic resonance spectroscopy (NMR) and X-ray crystallographic analysis. The determination of epitopes using X-ray techniques requires the protein-antibody complex to be crystallized, whereas NMR allows analysis of the complex in a liquid state. NMR measures the amount of amino acids as well as the neighbourhood of protons of different amino acid residues, wherein the alternating effect of two protons along the carbon backbone is characteristic of a particular epitope.

10 A successful method to recognize non-conformational linear epitopes is the immunoblot and in particular, the Western blot. Peptides may be generated from a complete *Lawsonia spp.* polypeptide by digestion with site-specific proteases, such as trypsin or chymotrypsin, and the peptides generated thereby can be separated using standard electrophoretic or chromatographic procedures. For example, after  
15 electrophoresis according to molecular weight using SDS/PAGE (SDS/polyacrylamide gel electrophoresis) and/or according to isoelectric point using IEF (isoelectric focussing) or alternatively, by two-dimensional electrophoresis, the peptides can be transferred to immobilizing nylon or nitrocellulose membranes and incubated with sera raised against the intact polypeptides. Peptides that comprise immunogenic regions  
20 (i.e., B-cell or T-cell epitopes) are bound by the antibodies in the sera and the bound antibodies may be detected using secondary antibodies, such as anti-IgG antibodies, that have been labelled radioactively or enzymatically. The epitopes may then be characterised by purification based upon their size, charge or ability to bind specifically to antibodies against the intact polypeptide, using one or more techniques, such as  
25 size-exclusion chromatography, ion-exchange chromatography, affinity chromatography or ELISA among others. After purification of the epitope, only one band or spot should be detectable with gel electrophoresis. The N-terminal or total sequencing of the polypeptide or peptide fragment offers the possibility to compare the amino acid sequence with known proteins in databases.

30

Several computer-driven algorithms have now been devised to search for T-cell epitopes in proteins (Margalit *et al*, 1987; Vajda and C. DeLisi, 1990; Altuvia *et al.*,

1994; Parker *et al.* 1994; DeGroot *et al.*, 1995; Gabriel *et al.*, 1995; Meister *et al.*, 1995). These algorithms search the amino acid sequence of a given protein for characteristics believed to be common to immunogenic peptides, locating regions that are likely to induce a cellular immune response *in vitro*. Computer-driven algorithms can identify  
5 regions of a *Lawsonia spp.* polypeptide that contain epitopes and are less variable among different isolates. Alternatively, computer-driven algorithms can rapidly identify regions of each isolate's more variable proteins that should be included in a multivalent vaccine.

10 The AMPHI algorithm (Margalit *et al.*, 1987), which is based on the periodicity of T cell epitopes, has been widely used for the prediction of T-cell antigenic sites from sequence information alone. Essentially, AMPHI describes a common structural pattern of MHC binding motifs, since MHC binding motifs (i.e., patterns of amino acids that appear to be common to most of the peptides that bind to a specific MHC  
15 molecule) appear to exhibit the same periodicity as an alpha helix. Identification of T-cell epitopes by locating MHC binding motifs in an amino acid sequence provides an effective means of identifying immunogenic epitopes in diagnostic assays.

The EpiMer algorithm (Meister *et al.*, 1995; Gabriel *et al.*, 1995; DeGroot *et al.*, 1995)  
20 locates clustered MHC binding motifs in amino acid sequences of proteins, based upon the correlation between MHC binding motif-dense regions and peptides that may have the capacity to bind to a variety of MHC molecules (promiscuous or multi-determinant binders) and to stimulate an immune response in these various MHC contexts as well (promiscuous or multi-determinant epitopes). The EpiMer algorithm  
25 uses a library of MHC binding motifs for multiple class I and class II HLA alleles to predict antigenic sites within a protein that have the potential to induce an immune response in subjects with a variety of genetic backgrounds. EpiMer locates matches to each MHC-binding motif within the primary sequence of a given protein antigen. The relative density of these motif matches is determined along the length of the antigen,  
30 resulting in the generation of a motif-density histogram. Finally, the algorithm identifies protein regions in this histogram with a motif match density above an algorithm-defined

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cutoff density value, and produces a list of subsequences representing these clustered, or motif-rich regions. The regions selected by EpiMer may be more likely to act as multi-determinant binding peptides than randomly chosen peptides from the same antigen, due to their concentration of MHC-binding motif matches. The selection  
5 of regions that are MHC binding motif-dense increases the likelihood that the predicted polypeptide or peptide fragment contains a "valid" motif, and furthermore, that the reiteration of identical motifs may contribute to binding.

Additional MHC binding motif-based algorithms have been described by Parker *et al.*(1994) and Altuvia *et al.*(1994). In these algorithms, binding to a given MHC  
10 molecule is predicted by a linear function of the residues at each position, based on empirically defined parameters, and in the case of the Altuvia *et al.*(1994) algorithm, known crystallographic structures may also be taken into consideration.

15 Recombinant methods offer the opportunity to obtain well characterized epitopes of high purity for the production of diagnostic reagents and epitope-specific vaccine formulations (Mohapatra *et al.*, 1995). Based upon the amino acid sequence of a linear epitope and identification of the corresponding nucleotide sequence encoding same, polymerase chain reaction (PCR) may be performed to amplify the epitope-encoding  
20 region from cDNA. After cloning and expression in a suitable vector/host system, a large amount of epitopes of high purity can be extracted. Accordingly, the present invention clearly extends to both isolated non-recombinant polypeptides and recombinant polypeptides in an impure or isolated form.

25 The term "polypeptide" as used herein shall be taken to refer to any polymer consisting of amino acids linked by covalent bonds and includes within its scope the full-length amino acids disclosed herein, and any parts or fragments thereof such as, for example, peptides consisting of about 5-50 amino acid residues in length, preferably about 5-30 amino acid residues in length, more preferably about 5-20 amino acid  
30 residues in length, and even more preferably about 5-10 amino acid residues in length. Also included within the scope of the definition of a "polypeptide" are amino acid

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sequence variants, containing one or more preferably conservative amino acid substitutions, deletions, or insertions, which do not alter at least one essential property of said polypeptide such as, for example, its immunogenicity, use as a diagnostic reagent, or effectiveness as a vaccine against *Lawsonia spp*, amongst others.

- 5 Accordingly, a polypeptide may be isolated from a source in nature, or chemically synthesized. Furthermore, a polypeptide may be derived from a full-length protein by chemical or enzymatic cleavage, using reagents such as CNBr, trypsin, or chymotrypsin, amongst others.
- 10 Conservative amino acid substitutions are well-known in the art. For example, one or more amino acid residues of a native flagellar hook protein of the present invention can be substituted conservatively with an amino acid residue of similar charge, size or polarity, with the resulting polypeptide retaining an ability to function in a vaccine or as a diagnostic reagent as described herein. Rules for making such substitutions include
- 15 those described by Dayhof (1978). More specifically, conservative amino acid substitutions are those that generally take place within a family of amino acids that are related in their side chains. Genetically-encoded amino acids are generally divided into four groups: (1) acidic=aspartate, glutamate; (2) basic=lysine, arginine, and histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine,
- 20 and tryptophan; and (4) uncharged polar= glycine, asparagine, glutamine, cysteine, serine, threonine, and tyrosine. Phenylalanine, tyrosine and tryptophan are also jointly classified as aromatic amino acids. One or more replacements within any particular group such as, for example, the substitution of leucine for isoleucine or valine or alternatively, the substitution of aspartate for glutamate or threonine for serine, or of
- 25 any other amino acid residue with a structurally-related amino acid residue, will generally have an insignificant effect on the function of the resulting polypeptide.

The present invention is not limited by the source of the subject immunogen and clearly extends to isolated and recombinant polypeptides which are derived from a

30 natural or a non-natural occurring source.

The term "recombinant polypeptide" as used herein shall be taken to refer to a polypeptide which is produced *in vitro* or in a host cell by the expression of a genetic sequence encoding said polypeptide, which genetic sequence is under the control of a suitable promoter, wherein a genetic manipulation has been performed in order to achieve said expression. Accordingly, the term "recombinant polypeptide" clearly encompasses polypeptides produced by the expression of genetic sequences contained in viral vectors, cosmids or plasmids that have been introduced into prokaryotic or eukaryotic cells, tissues or organs. Genetic manipulations which may be used in this context will be known to those skilled in the art and include, but are not limited to, nucleic acid isolation, restriction endonuclease digestion, exonuclease digestion, end-filling using the Klenow fragment of *E. coli* DNA polymerase I or T4 DNA polymerase enzymes, blunt-ending of DNA molecules using T4 DNA polymerase or ExoIII enzymes, site-directed mutagenesis, ligation, and amplification reactions. As will be known to those skilled in the art, additional techniques such as nucleic acid hybridisations and nucleotide sequence analysis may also be utilised in the preparation of recombinant polypeptides, in confirming the identity of a nucleic acid molecule encoding a desired recombinant polypeptide and a genetic construct comprising the nucleic acid molecule.

Wherein the polypeptide of the present invention is a recombinant polypeptide, it may be produced in and, if desirable, isolated from a recombinant viral vector expression system or host cell. As will be known to those skilled in the relevant art, a cell for production of a recombinant polypeptide is selected on the basis of several parameters including the genetic constructs used to express the polypeptide under consideration, as well as the stability and activity of said polypeptide. It will also be known to those skilled in the art that the stability or activity of a recombinant polypeptide may be determined, at least in part, by post-translational modifications to the polypeptide such as, for example, glycosylation, acylation or alkylation reactions, amongst others, which may vary between cell lines used to produce the recombinant polypeptide.

30

Accordingly, in a more particularly preferred embodiment, the present invention extends to a recombinant polypeptide or a derivative, homologue or analogue thereof

as present in a virus particle, or as produced in prokaryotic or eukaryotic host cell, or in a virus or cell culture thereof.

5 The present invention also extends to a recombinant polypeptide according to any of the foregoing embodiments which is produced in a bacterial cell belonging to the genus *Lawsonia*, in particular a cell of *L. intracellularis*, or a culture thereof.

10 The term "isolated polypeptide" refers to a polypeptide of the present invention which has been purified to some extent, preferably to at least about 20% by weight of protein, preferably to at least about 50% by weight of protein, more preferably to at least about 60% by weight of protein, still more preferably to at least about 70% by weight of protein and even more preferably to at least about 80% by weight of protein or greater, from its natural source or, in the case of non-naturally-occurring polypeptides, from the culture medium or cellular environment in which it was produced. Such isolation may  
15 be performed to improve the immunogenicity of the polypeptide of the present invention, or to improve the specificity of the immune response against that polypeptide, or to remove toxic or undesirable contaminants therefrom. The necessary or required degree of purity of an isolated polypeptide will vary depending upon the purpose for which the polypeptide is intended, and for many applications it will be  
20 sufficient for the polypeptide preparation to contain no contaminants which would reduce the immunogenicity of the polypeptide when administered to a host animal, in particular a porcine or avian animal being immunized against PPE or, alternatively, which would inhibit immuno-specific binding in an immunoassay for the diagnosis of PPE or a causative agent thereof.

25

The purity of an isolated polypeptide of the present invention may be determined by any means known to those skilled in the art, including the degree of homogeneity of a protein preparation as assessed by SDS/polyacrylamide gel electrophoresis, 2-dimensional electrophoresis, or amino acid composition analysis or sequence analysis.

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Preferably, the polypeptide of the present invention will be substantially homogeneous or substantially free of nonspecific proteins, as assessed by SDS/polyacrylamide gel

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electrophoresis, 2-dimensional electrophoresis, or amino acid composition analysis or sequence analysis.

5 The polypeptide of the present invention can be purified for use as a component of a vaccine composition by any one or a combination of methods known to those of ordinary skill in the art, including, for example, reverse phase chromatography, HPLC, ion-exchange chromatography, and affinity chromatography, among others.

10 In a preferred embodiment, the isolated or recombinant polypeptide of the invention functions is secretable into the periplasmic space of a cell, preferably into the periplasm of a prokaryotic cell, such as, for example, *Escherichia coli*. or *L. intracellularis*, or, alternatively, is immunologically cross-reactive with a *L. intracellularis* polypeptide selected from the group consisting of flhB, flhR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN.

15 In a particularly preferred embodiment, the isolated or recombinant polypeptide of the invention is derived from *Lawsonia spp.* or other pathogenic agent associated with the onset and/or development of PPE and more preferably, the subject polypeptide is derived from *L. intracellularis*.

20 A B-cell or T-cell epitope of a polypeptide selected from the group consisting of flhB, flhR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue or derivative of any one or more of said polypeptides, may comprise one or more of the following:

- 25
- (i) the primary amino acid sequence of any one of said polypeptides, as determined by an art-accepted methodology to comprise a continuous non-conformational epitope;
  - (ii) the secondary structure which any one of said polypeptides adopt, as
- 30
- determined by an art-accepted methodology to comprise a continuous conformational epitope;

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- (iii) the tertiary structure which any one of said polypeptides adopt in contact with another region of the same polypeptide molecule, as determined by an art-accepted methodology to comprise a discontinuous conformational epitope; or
- 5 (iv) the quaternary structure which any one of said polypeptides adopt in contact with a region of another polypeptide molecule, as determined by an art-accepted methodology to comprise a discontinuous conformational epitope.

Accordingly, immunogenic polypeptides or derivatives, homologues or analogues  
10 thereof comprising the same, or substantially the same primary amino acid sequence are hereinafter defined as "immunogens which comprise a B-cell or T-cell epitope" or similar term.

Immunogenic polypeptides or derivatives, homologues, or analogues thereof  
15 comprising different primary amino acid sequences may comprise immunologically identical immunogens, because they possess conformational B-cell or T-cell epitopes that are recognised by the immune system of a host species to be identical. Such immunogenic polypeptides or derivatives, homologues or analogues thereof are hereinafter defined as "immunogens which mimic or cross-react with a B-cell or T-cell  
20 epitope", or similar term.

Accordingly, the present invention extends to an immunogen which comprises, mimics, or cross-reacts with a B-cell or T-cell epitope of an isolated or recombinant polypeptide according to any one of the foregoing embodiments or a derivative, homologue or  
25 analogue thereof. In a particularly preferred embodiment, the present invention provides an immunogen which comprises, mimics, or cross-reacts with a B-cell or T-cell epitope of an isolated or recombinant polypeptide which in its native form is obtainable from a species of *Lawsonia* such as, but not limited to *L. intracellularis* and which polypeptide preferably has the same biological function as a polypeptide  
30 selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN, as hereinbefore defined.

Preferably, such immunogenic polypeptides will not comprise a primary amino acid sequence which is highly-conserved between *L. intracellularis* and another non-pathogenic microorganism which is normally resident in the gut or other organ of an animal, in particular a porcine or avian animal. The significance of this exclusion to those embodiments of the invention wherein specificity is essential to performance (eg vaccine and diagnostic applications) will be apparent to those skilled in the art.

To improve the immunogenicity of a subject polypeptide of the present invention one or more amino acids not corresponding to the original protein sequence can be added to the amino or carboxyl terminus of the polypeptide. Such extra amino acids are useful for coupling the polypeptide to another peptide or polypeptide, to a large carrier protein or to a solid support. Amino acids that are useful for these purposes include but are not limited to tyrosine, lysine, glutamic acid, aspartic acid, cysteine and derivatives thereof. Additional protein modification techniques can be used such as, e.g., NH<sub>2</sub>-acetylation or COOH-terminal amidation, to provide additional means for coupling the polypeptide to another polypeptide or peptide molecule, or to a solid support. Procedures for coupling polypeptides to each other, or to carrier proteins or solid supports, are well known in the art. Polypeptides containing the above-mentioned extra amino acid residues at either the carboxyl- or amino-termini and either uncoupled or coupled to a carrier or solid support, are consequently within the scope of the present invention.

Furthermore, the polypeptide can be immobilised to a polymeric carrier or support material.

In an alternative embodiment, the immunogenicity of a polypeptide of the present invention may be improved using molecular biology techniques to produce a fusion protein containing one or more polypeptides of the present invention fused to a carrier molecules such as a highly immunogenic protein. For example, a fusion protein containing a polypeptide of the present invention fused to the highly immunogenic B subunit of cholera toxin can be used to increase the immune response to the

polypeptide. The present invention also contemplates fusion proteins comprising a cytokine, such as an interleukin, fused to the subject polypeptide of the present invention, and genes encoding same.

- 5 Preferably, the polypeptide of the present invention, or a derivative, homologue or analogue thereof, when administered to a mammal, induces an immune response in said mammal. More preferably, the polypeptide of the present invention, when administered to a mammal, in particular a porcine animal (e.g., a pig) induces a protective immune response against *Lawsonia spp.*, and preferably against *L.*  
10 *intracellularis*, therein. As used herein, the phrase "induction of a protective immune response", and the like, refers to the ability of the administered polypeptide of the present invention to prevent or detectably slow the onset, development, or progression of symptoms associated with *Lawsonia* infection, and preferably, to prevent or detectably slow the onset, development, or progression of symptoms associated with  
15 PPE in pigs.

Preferably, the isolated or recombinant immunogenic polypeptide is selected from the group consisting of the following:

- (i) a polypeptide which comprises an amino acid sequence which has at  
20 least about 60% sequence identity overall to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;
- (ii) a polypeptide which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group  
25 consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);
- (iii) a polypeptide which comprises at least about 5 contiguous amino acids,  
30 preferably at least about 10 contiguous amino acids and more preferably at

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least about 20 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

- 5 (iv) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and
- 10 (v) a homologue, analogue or derivative of any one of (i) to (iv) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

In an alternative preferred embodiment, the isolated or recombinant immunogenic polypeptide is selected from the group consisting of the following:

- 15 (i) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;
- (ii) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to the nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);
- 20 (iii) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;
- 25 (iv) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence of *L. intracellularis* DNA contained within a plasmid
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selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and  
5 NM00/16482 (plasmid pGTE#7 ytfM); and

(v) a homologue, analogue or derivative of any one of (i) to (iv) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

Preferably, the immunogenic polypeptide encompassed by the present invention has  
10 at least about 70% identity, more preferably at least about 80% identity, even more preferably at least about 90% identity, and still even more preferably at least about 95% identity to the amino acid sequence of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined.

15 In determining whether or not two amino acid sequences fall within these percentage limits, those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or alignments, differences will arise in the positioning of non-identical residues, depending upon the  
20 algorithm used to perform the alignment. In the present context, reference to a percentage sequence identity or similarity between two or more amino acid sequences shall be taken to refer to the number of identical and similar residues respectively, between said sequences as determined using any standard algorithm known to those skilled in the art. For example, amino acid sequence identities or similarities may be  
25 calculated using the GAP programme of the Computer Genetics Group, Inc., University Research Park, Madison, Wisconsin, United States of America (Devereaux *et al*, 1984). The GAP programme utilizes the algorithm of Needleman and Wunsch (1970) to maximise the number of identical/similar residues and to minimise the number and/or length of sequence gaps in the alignment. Alternatively or in addition, where  
30 more than two amino acid sequences are being compared, the ClustalW programme of Thompson *et al* (1994) can be used.

Preferably, the isolated or recombinant immunogenic polypeptide of the invention comprises at least about 10 contiguous amino acids of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined. More preferably, the isolated or recombinant immunogenic polypeptide of the invention comprises at least about 20 contiguous amino acid residues of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined. Even more preferably, the isolated or recombinant immunogenic polypeptide of the invention comprises at least about 30 contiguous amino acid residues of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined, and still even more preferably, at least about 40 contiguous amino acid residues of said flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, or ytfN polypeptides.

The present invention further encompasses homologues, analogues and derivatives of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined.

"Homologues" of a polypeptide are those immunogenic polypeptides that are derived from a full-length *L. intracellularis* polypeptides described herein, or have sequence similarity to a full-length *L. intracellularis* polypeptide, notwithstanding one or more amino acid substitutions, deletions and/or additions relative to the full-length *L. intracellularis* polypeptide. A homologue may also retain the biological activity or catalytic activity of the full-length polypeptide. In such homologues, one or more amino acids can be replaced by other amino acids having similar properties such as, for example, hydrophobicity, hydrophilicity, hydrophobic moment, antigenicity, propensity to form or break  $\alpha$ -helical structures of  $\beta$ -sheet structures, and so on.

Substitutional variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Amino acid substitutions

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are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1-10 amino acid residues. and deletions will range from about 1-20 residues. Preferably, amino acid substitutions will comprise conservative amino acid substitutions, such as those described *supra*.

Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein. Insertions can comprise amino-terminal and/or carboxyl terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino or carboxyl terminal fusions, of the order of about 1 to 4 residues.

Deletional variants are characterised by the removal of one or more amino acids from the sequence.

Amino acid variants of the polypeptide of the present invention may readily be made using polypeptide synthetic techniques well known in the art, such as solid phase synthesis and the like, or by recombinant DNA manipulations. The manipulation of DNA sequences to produce variant proteins which manifest as substitutional, insertional or deletional variants are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA having known sequence are well known to those skilled in the art, such as by M13 mutagenesis or other site-directed mutagenesis protocol.

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"Analogues" are defined as those immunogenic polypeptides that are derived from a full-length *L. intracellularis* polypeptides described herein, or have sequence similarity to a full-length *L. intracellularis* polypeptide; notwithstanding one or more non-naturally occurring or modified amino acid residues relative to the naturally-occurring full-length *L. intracellularis* polypeptide. The term "analogue" shall also be taken to include an amino acid sequence which is not similar to an amino acid sequence of a full-length

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*L. intracellularis* polypeptide set forth herein, however mimics or cross-reacts with a B-cell or T-cell epitope of *Lawsonia spp.* and preferably, mimics or cross-reacts with a B-cell or T-cell epitope of *L. intracellularis*, such as, for example, a polypeptide which is derived from a computational prediction or empirical data revealing the secondary, tertiary or quaternary structure of the full-length polypeptide or an epitope thereof.

For example, mimotopes (polypeptide analogues that cross-react with a B-cell or T-cell epitope of the *Lawsonia* polypeptide of the invention but, however, comprise a different amino acid sequence to said epitope) may be identified by screening random amino acid sequences in polypeptide libraries with antibodies that bind to a desired T-cell or B-cell epitope. As with techniques for the identification of B-cell or T-cell epitopes as described *supra*, the antibodies used to identify such mimotopes may be polyclonal or monoclonal or recombinant antibodies, in crude or purified form. Mimotopes of a T-cell epitope may then be assayed further for their ability to stimulate T-cell cytotoxic or proliferative responses *in vitro*. Mimotopes are particularly useful as analogues of non-linear (i.e., conformational) epitopes of the polypeptide of the present invention, because conformational epitopes are generally formed from non-contiguous regions in a polypeptide, and the mimotopes provide immunogenic equivalents thereof in the form of a single polypeptide molecule.

Additionally, the use of polypeptide analogues can result in polypeptides with increased immunogenic and/or antigenic activity, that are less sensitive to enzymatic degradation, and which are more selective. A suitable proline analogue is 2-aminocyclopentane carboxylic acid ( $\beta$ AC<sup>5</sup>c) which has been shown to increase the immunogenic activity of a native polypeptide more than 20 times (Mierke *et al*, 1990; Portoghese *et al*, 1990; Goodman *et al*, 1987).

"Derivatives" of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined, are those peptides or polypeptides which comprise at least about five contiguous amino acid residues of any one or more of said flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, or ytfN polypeptides.

A "derivative" may further comprise additional naturally-occurring, altered glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a flhB, or fliR, or ntrC, or glnH, or motA, or motB, or tlyC, or ytfM, or ytfN polypeptide, as hereinbefore defined. Alternatively or in addition, a derivative may comprise one or more non-amino acid substituents such as, for example, a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence of a flhB, or fliR, or ntrC, or glnH, or motA, or motB, or tlyC, or ytfM, or ytfN polypeptide, such as, for example, a reporter molecule which is bound thereto to facilitate its detection.

Other examples of recombinant or synthetic mutants and derivatives of a polypeptide immunogen of the present invention include those incorporating single or multiple substitutions in the amino acid sequence of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides. Recombinant or synthetic mutants and derivatives produced by making deletions from the amino acid sequence of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, are also included within the scope of preferred derivatives. Additionally, recombinant or synthetic mutants and derivatives produced by making additions to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, such as, for example, using carbohydrates, lipids and/or proteins or polypeptides, are also encompassed by the present invention.

Naturally-occurring or altered glycosylated or acylated forms of the flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, or ytfN polypeptides are particularly contemplated by the present invention.

Additionally, homopolymers or heteropolymers comprising one or more copies of the reference polypeptides, or one or more derivatives, homologues or analogues thereof, are clearly within the scope of the present invention.

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Preferably, homologues, analogues and derivatives of the flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, or ytfN polypeptides of the invention are "immunogenic", defined hereinafter as the ability of said polypeptide, or a derivative, homologue or analogue thereof, to elicit B cell and/or T cell responses in the host, in response to immunization.

5

Preferred homologues, analogues and derivatives of the flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, or ytfN polypeptides of the invention include any amino acid variant that functions as B cell or T cell epitope of any one of said polypeptides, wherein said variant is capable of mediating an immune response, such as, for example, a mimotope of the immunogenic polypeptide which has been produced by synthetic means, such as by Fmoc chemistry. The only requirement of such variant molecules is that they cross-react immunologically with a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN, as hereinbefore defined, or an epitope of said polypeptide.

15

As will be apparent to those skilled in the art, such homologues, analogues and derivatives of the polypeptides of the invention molecules will be useful to prepare antibodies that cross-react with antibodies against said polypeptide and/or to elicit a protective immune response of similar specificity to that elicited by said polypeptide. Such molecules will also be useful in diagnostic and other applications that are immunological in nature such as, for example, diagnostics which utilise one or more immunoassay formats (eg. ELISA, RIA and the like).

20

Accordingly, the immunogen of the present invention or a derivative, homologue or analogue thereof is useful in vaccine compositions that protect an individual against infection by *L. intracellularis* and/or as an antigen to elicit polyclonal or monoclonal antibody production and/or in the detection of antibodies against *L. intracellularis* in infected animals, particularly in porcine and avian animals.

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The polypeptides of the present invention may comprise leader sequences to facilitate their secretion into the periplasmic space, either as part of the native protein, or alternatively, added by recombinant engineering means. Such may have improved

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immunogenicity compared to non-secreted or non-secretable polypeptides of *L. intracellularis*, or non-secreted or non-secretable polypeptides of other causative agents of PPE. The particular advantages of such peptides will be immediately apparent to those skilled in the production of vaccine compositions, where the inherent immunogenicity of the immunogen is an important consideration for a protective immune response to be conferred.

Moreover, unique regions of the *L. intracellularis* polypeptides exemplified herein are promising antigenic peptides for the formulation of *Lawsonia*-specific vaccines and diagnostics for the specific detection of *Lawsonia* spp. in biological samples.

A second aspect of the present invention provides a vaccine composition for the prophylaxis or treatment of infection in a mammal or bird by *L. intracellularis* or similar or otherwise related microorganism, said vaccine composition comprising:

- (i) an immunogenic component which comprises an isolated or recombinant polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides or an immunogenic homologue, analogue or derivative of any one of said polypeptides which is immunologically cross-reactive with *L. intracellularis*; and
- (ii) one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

As used herein, the term "immunogenic component" refers to a polypeptide encoded by DNA from, or derived from, *L. intracellularis* or a related microorganism thereto which is capable of inducing a protective immune response in an animal, in particular a porcine or avian animal, whether or not said polypeptide is in an isolated or recombinant form. Accordingly, the vaccine composition clearly encompasses those vaccine compositions which comprise attenuated, killed or non-pathogenic isolates or forms of *L. intracellularis* or related microorganisms thereto which comprise or express said polypeptide.

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By "protective immune response" is meant that the immunogenic component elicits an immune response in the animal to which the vaccine composition is administered at the humoral and/or cellular level which is sufficient to prevent infection by *L. intracellularis* or a related microorganism thereto and/or which is sufficient to detectably  
5 reduce one or more symptoms or conditions, or to detectably slow the onset of one or more symptoms or conditions, associated with infection by *L. intracellularis* or a related microorganism thereto in an animal host, as compared to a control infected animal.

The term "effective amount" of an immunogenic component present in the vaccine composition refers to that amount of said immunogenic component that is capable of  
10 inducing a protective immune response after a single complete dose has been administered, or after several divided doses have been administered.

Preferably, the polypeptide component of the subject vaccine composition comprises an amino acid sequence which is both immunogenic and specific, by virtue of its  
15 immunological cross-reactivity with the causative agent of PPE, *L. intracellularis*. In this regard, it will be apparent from the preceding description that such polypeptide components may comprise the amino acid sequence of a polypeptide of *L. intracellularis* as exemplified herein, or alternatively, an immunologically cross-reactive homologue, analogue or derivative of said amino acid sequence, such as, for example,  
20 a mimotope of said sequence.

The immunogenic polypeptide or immunogenic homologue, analogue or derivative may be a naturally-occurring polypeptide in isolated or recombinant form according to any of the embodiments described *supra* or exemplified herein. Preferably, the  
25 immunogenic polypeptide or immunogenic homologue, analogue or derivative is derived from *Lawsonia spp.*, in particular *L. intracellularis* or a microorganism that is related thereto.

Preferably, the immunogenic component has undergone at least one purification step  
30 or at least partial concentration from a cell culture comprising *L. intracellularis* or a related microorganism thereto, or from a lysed preparation of *L. intracellularis* cells or

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related microorganism, or from another culture in which the immunogenic component is recombinantly expressed. The purity of such a component which has the requisite immunogenic properties is preferably at least about 20% by weight of protein in a particular preparation, more preferably at least about 50%, even more preferably at least about 60%, still more preferably at least about 70% and even more preferably at least about 80% or greater.

The immunogenic component of the vaccine of the present invention can comprise a single polypeptide, or a range or combination of different polypeptides covering different or similar epitopes. In addition or, alternatively, a single polypeptide can be provided with multiple epitopes. The latter type of vaccine is referred to as a polyvalent vaccine. A multiple epitope includes two or more epitopes located within a polypeptide molecule.

The formulation of vaccines is generally known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pennsylvania, USA.

A particularly useful form of the vaccine is a recombinant vaccine produced, for example, in a vaccine vector, such as but not limited to a mammalian cell transfected with a vaccinia virus vector, an insect cell transfected with a baculovirus vector, or a bacterial cell transfected with a plasmid or cosmid, the only requirement being that the vector expresses the immunogenic component.

The present invention clearly extends to recombinant vaccine compositions in which the immunogenic component at least is contained within killed vaccine vectors prepared, for example, by heat, formalin or other chemical treatment, electric shock or high or low pressure forces. According to this embodiment, the immunogenic component of the vaccine is generally synthesized in a live vaccine vector which is killed prior to administration to an animal.

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Furthermore, the vaccine vector expressing the immunogenic component may be non-pathogenic or attenuated. Within the scope of this embodiment are cells that have been transfected with non-pathogenic or attenuated viruses encoding the immunogenic component of the vaccine and non-pathogenic or attenuated cells that  
5 directly express the immunogenic component.

Attenuated or non-pathogenic host cells include those cells which are not harmful to an animal to which the subject vaccine is administered. As will be known to those skilled in the art, "live vaccines" can comprise an attenuated virus vector encoding the  
10 immunogenic component or a host cell comprising same, which is capable of replicating in an animal to which it is administered, and using host cell machinery to express the immunogenic component albeit producing no adverse side-effects therein. Such vaccine vectors may colonise the gut or other organ of the vaccinated animal. Such live vaccine vectors are efficacious by virtue of their ability to continually express  
15 the immunogenic component in the host animal for a time and at a level sufficient to confer protective immunity against a pathogen which expresses an immunogenic equivalent of said immunogenic component. The present invention clearly encompasses the use of such attenuated or non-pathogenic vectors and live vaccine preparations.

20

The vaccine vector may be a virus, bacterial cell or a eukaryotic cell such as an insect, avian, porcine or other mammalian cell or a yeast cell or a cell line such as COS, VERO, HeLa, mouse C127, Chinese hamster ovary (CHO), WI-38, baby hamster kidney (BHK) or MDCK cell lines. Suitable prokaryotic cells include *Mycobacterium*  
25 *spp.*, *Corynebacterium spp.*, *Salmonella spp.*, *Escherichia coli*, *Bacillus spp.* and *Pseudomonas spp.*, amongst others. Bacterial strains which are suitable for the present purpose are well-known in the relevant art (Ausubel *et al*, 1987; Sambrook *et al*, 1989).

30 Such cells and cell lines are capable of expression of a genetic sequence encoding a polypeptide of the present invention from *L. intracellularis*, or a homologue, analogue

or derivative thereof, in a manner effective to induce a protective immune response in the animal. For example, a non-pathogenic bacterium can be prepared containing an expression vector which comprises a nucleotide sequence encoding a polypeptide selected from the group consisting of *flhB*, *fliR*, *ntnC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* polypeptides, or a homologue, analogue, or derivative thereof, wherein said nucleotide sequence is placed operably under the control of a constitutive or inducible promoter sequence. The bacterium is then permitted to colonise suitable locations in a pig's gut, where it replicates and expresses the said polypeptide in amount sufficient to induce a protective immune response against *L. intracellularis*.

In a further alternative embodiment, the vaccine can be a DNA or RNA vaccine comprising a DNA or RNA molecule encoding a polypeptide selected from the group consisting of *flhB*, *fliR*, *ntnC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* polypeptides or homologues, analogues or derivatives thereof, wherein said vaccine is injected into muscular tissue or other suitable tissue in a pig under conditions sufficient to permit transient expression of said DNA or RNA to produce an effective amount of said polypeptide to induce a protective immune response. In a preferred embodiment, the DNA vaccine is in the form of a plasmid, in which the DNA is operably connected with a promoter region capable of expressing the nucleotide sequence encoding the immunogen in cells of the immunized animal.

In the production of a recombinant vaccine, except for a DNA vaccine described herein, it is therefore necessary to express the immunogenic component in a suitable vector system. For the present purpose, the immunogenic component can be expressed by:

- (i) placing an isolated nucleic acid molecule in an expressible format, said nucleic acid molecule comprising the coding region of a gene selected from the group consisting of *flhB*, *fliR*, *ntnC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes, or a protein-encoding homologue, analogue or derivative thereof;
- (ii) introducing the isolated nucleic acid molecule of (i) in an expressible format into a suitable vaccine vector; and

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(iii) incubating or growing the vaccine vector for a time and under conditions sufficient for expression of the immunogenic component encoded by said nucleic acid molecule to occur.

- 5 It will be apparent from the preceding discussion that the protein-encoding region of a *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, or *ytfN* gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, and 17, or alternatively or in addition, a protein-encoding nucleotide sequence of *L. intracellularis* DNA contained within a deposited plasmid selected from the group
- 10 consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 *glnH*); NM00/16477 (plasmid pGTE#2 *flhB*); NM00/16478 (plasmid pGTE#3 *fliR*); NM00/16479 (plasmid pGTE#4 *motA/B*); NM00/16480 (plasmid pGTE#5 *tlyC*); NM00/16481 (plasmid pGTE#6 *ntrC*); and NM00/16482 (plasmid pGTE#7 *ytfM*).
- 15 Preferred homologues of the protein-encoding region of a *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, or *ytfN* gene include those nucleotide sequences selected from the group consisting of:
- (i) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group
- 20 consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17 or a degenerate variant thereof;
- (ii) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to the protein-encoding sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL
- 25 Accession Nos: NM00/16476 (plasmid pGTE#1 *glnH*); NM00/16477 (plasmid pGTE#2 *flhB*); NM00/16478 (plasmid pGTE#3 *fliR*); NM00/16479 (plasmid pGTE#4 *motA/B*); NM00/16480 (plasmid pGTE#5 *tlyC*); NM00/16481 (plasmid pGTE#6 *ntrC*); and NM00/16482 (plasmid pGTE#7 *ytfM*);
- (iii) a protein-encoding nucleotide sequence which comprises at least about
- 30 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

- (iv) a protein-encoding nucleotide sequence which comprises at least about 15 contiguous nucleotides of the protein-encoding sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);
- (v) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the complement of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17; and
- (vi) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the non-coding strand of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

The present invention clearly extends to analogues or derivatives of any one of (i) to (vi) which encode a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

For the present purpose, a preferred homologue of the protein-encoding region of a *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, or *ytfN* gene will have at least about 80% nucleotide sequence identity to the coding region of said gene, still more preferably at least about 90% identity, and yet still more preferably at least about 95% identity.

In determining whether or not two nucleotide sequences fall within these percentage limits, those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or alignments, differences may arise in the positioning of non-identical residues, depending upon the

- algorithm used to perform the alignment. In the present context, reference to a percentage identity between two or more nucleotide sequences shall be taken to refer to the number of identical residues between said sequences as determined using any standard algorithm known to those skilled in the art. For example, nucleotide sequences may be aligned and their identity calculated using the BESTFIT programme or other appropriate programme of the Computer Genetics Group, Inc., University Research Park, Madison, Wisconsin, United States of America (Devereaux *et al*, 1984).
- 10 Preferably, a homologue of the protein-encoding region of a *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, or *ytfN* gene hybridizes under at least medium stringency conditions to the non-coding strand of said gene, even more preferably under high stringency conditions to the non-coding strand of said gene.
- 15 For the purposes of defining the level of stringency, a low stringency is defined herein as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. A moderate stringency is defined herein as being a hybridisation and/or washing carried out in 2xSSC buffer, 0.1% (w/v) SDS at a temperature in the range 45°C to 65°C. A high stringency is defined herein as being a hybridisation and/or
- 20 wash carried out in 0.1xSSC buffer, 0.1% (w/v) SDS, or lower salt concentration, and at a temperature of at least 65°C. Reference herein to a particular level of stringency encompasses equivalent conditions using wash/hybridization solutions other than SSC known to those skilled in the art.
- 25 Generally, the stringency is increased by reducing the concentration of SSC buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. Those skilled in the art will be aware that the conditions for hybridisation and/or wash may vary depending upon the nature of the hybridisation membrane or the type of hybridisation probe used. Conditions for hybridisations and
- 30 washes are well understood by one normally skilled in the art. For the purposes of clarification of the parameters affecting hybridisation between nucleic acid molecules,

reference is found in pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

5 As used herein, a "nucleic acid molecule in an expressible format" is a protein-encoding region of a nucleic acid molecule placed in operable connection with a promoter or other regulatory sequence capable of regulating expression in the vaccine vector system.

10 Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e., upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. In the present context, the term  
15 "promoter" is also used to describe a recombinant, synthetic or fusion molecule, or derivative which confers, activates or enhances the expression of a nucleic acid molecule to which it is operably connected, and which encodes the immunogenic polypeptide. Preferred promoters can contain additional copies of one or more specific regulatory elements to further enhance expression and/or to alter the spatial  
20 expression and/or temporal expression of the said nucleic acid molecule.

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Placing a nucleic acid molecule under the regulatory control of, i.e., "in operable connection with", a promoter sequence means positioning said molecule such that expression is controlled by the promoter sequence. Promoters are generally, but not necessarily, positioned 5' (upstream) to the genes that they control. In the construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the genes from which it is derived. Again, as is known in the art, some variation in this distance can also occur.

The prerequisite for producing intact polypeptides in bacteria such as *E. coli* is the use of a strong promoter with an effective ribosome binding site. Typical promoters suitable for expression in bacterial cells such as *E. coli* include, but are not limited to, the *lacZ* promoter, temperature-sensitive  $\lambda_L$  or  $\lambda_R$  promoters, T7 promoter or the IPTG-inducible *tac* promoter. A number of other vector systems for expressing the nucleic acid molecule of the invention in *E. coli* are well-known in the art and are described, for example, in Ausubel *et al* (1987) or Sambrook *et al* (1989). Numerous plasmids with suitable promoter sequences for expression in bacteria and efficient ribosome binding sites have been described, such as for example, pKC30 ( $\lambda_L$ : Shimatake and Rosenberg, 1981); pKK173-3 (*tac*: Amann and Brosius, 1985), pET-3 (T7: Studier and Moffat, 1986); the pBAD/TOPO or pBAD/Thio-TOPO series of vectors containing an arabinose-inducible promoter (Invitrogen, Carlsbad, CA), the latter of which is designed to also produce fusion proteins with thioredoxin to enhance solubility of the expressed protein; the pFLEX series of expression vectors (Pfizer Inc., CT, USA); or the pQE series of expression vectors (Qiagen, CA), amongst others. Typical promoters suitable

for expression in viruses of eukaryotic cells and eukaryotic cells include the SV40 late promoter, SV40 early promoter and cytomegalovirus (CMV) promoter, CMV IE (cytomegalovirus immediate early) promoter amongst others.

- 5 Means for introducing the isolated nucleic acid molecule or a genetic construct comprising same into a cell for expression of the immunogenic component of the vaccine composition are well-known to those skilled in the art. The technique used for a given organism depends on the known successful techniques. Means for introducing recombinant DNA into animal cells include microinjection, transfection mediated by
- 10 DEAE-dextran, transfection mediated by liposomes such as by using lipofectamine (Gibco, MD, USA) and/or cellfectin (Gibco, MD, USA), PEG-mediated DNA uptake, electroporation and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agracetus Inc., WI, USA) amongst others.
- 15 The immunogenic component of a vaccine composition as contemplated herein exhibits excellent therapeutic activity, for example, in the treatment and/or prophylaxis of PPE when administered in an amount which depends on the particular case. For example, for recombinant polypeptide molecules, from about 0.5  $\mu$ g to about 20 mg may be administered, preferably from about 1  $\mu$ g to about 10 mg, more preferably from
- 20 about 10  $\mu$ g to about 5 mg, and most preferably from about 50  $\mu$ g to about 1 mg equivalent of the immunogenic component in a volume of about 1ml to about 5ml. For DNA vaccines, a preferred amount is from about 0.1  $\mu$ g/ml to about 5 mg/ml in a volume of about 1 to about 5 ml. The DNA can be present in "naked" form or it can be administered together with an agent facilitating cellular uptake (e.g., in liposomes
- 25 or cationic lipids). The important feature is to administer sufficient immunogen to induce a protective immune response. The above amounts can be administered as stated or calculated per kilogram of body weight. Dosage regime can be adjusted to provide the optimum therapeutic response. For example, several divided doses can be administered or the dose can be proportionally reduced as indicated by the
- 30 exigencies of the therapeutic situation. Booster administration may also be required. The vaccine of the present invention can further comprise one or more additional

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immunomodulatory components such as, for example, an adjuvant or cytokine molecule, amongst others, that is capable of increasing the immune response against the immunogenic component. Non-limiting examples of adjuvants that can be used in the vaccine of the present invention include the RIBI adjuvant system (Ribi Inc.,  
5 Hamilton, MT, USA), alum, mineral gels such as aluminium hydroxide gel, oil-in-water emulsions, water-in-oil emulsions such as, for example, Block co-polymer (CytRx, Atlanta GA, USA), QS-21 (Cambridge Biotech Inc., Cambridge MA, USA), SAF-M (Chiron, Emeryville CA, USA), AMPHIGEN® adjuvant, Freund's complete adjuvant; Freund's incomplete adjuvant; and Saponin, QuilA or other saponin fraction,  
10 monophosphoryl lipid A, and Avridine lipid-amine adjuvant. Other immunomodulatory agents that can be included in the vaccine include, for example, one or more cytokines, such as interferon and/or interleukin, or other known cytokines. Non-ionic surfactants such as, for example, polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether may also be included in the vaccines of the present invention.

15 The vaccine composition can be administered in a convenient manner such as by oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or by implantation (eg., using slow release technology), provided that a sufficient degree of the immunogenicity of the immunizing  
20 antigen is retained for the purposes of eliciting an immune response in the animal being treated. Depending on the route of administration, the immunogenic component may be required to be coated in a material to protect it from the action of enzymes, acids and other natural conditions which may inactivate it, such as those in the digestive tract.

25 The vaccine composition may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof, or in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms. Alternatively, the  
30 vaccine composition can be stored in lyophilised form to be rehydrated with an appropriate vehicle or carrier prior to use.



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Pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be fluid to the extent that easy syringability exists, unless the pharmaceutical form is a solid or semi-solid such as when slow release technology is employed. In any event, it must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms.

- 10 The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.
- 15 The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents such as, for example,, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents such as, for example,, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the
- 20 compositions of agents delaying absorption such as, for example,, aluminum monostearate and gelatin.

- Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients
- 25 enumerated above, as required, followed by filter-sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients selected from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the
- 30 freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

The present invention extends to vaccine compositions which confer protection against infection by one or more isolates or sub-types of *L. intracellularis* including those that belong to the same serovar or serogroup as *L. intracellularis*. The vaccine composition preferably also confers protection against infection by other species of the genus *Lawsonia* or other microorganisms related thereto, as determined at the nucleotide, biochemical, structural, physiological and/or immunointeractive level; the only requirement being that said other species or other microorganism expresses a polypeptide which is immunologically cross-reactive to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue or derivative of any one or more of said polypeptides as described herein. For example, such related microorganisms may comprise genomic DNA which is at least about 70% identical overall to the genomic DNA of *L. intracellularis* as determined using standard genomic DNA hybridisation and analysis techniques.

The terms "serogroup" and "serovar" relate to a classification of microorganisms which is based upon serological typing data, in particular data obtained using agglutination assays such as the microscopic agglutination test (MAT). Those skilled in the art will be aware that serovar and serogroup antigens are a mosaic on the cell surface and, as a consequence there will be no strict delineation between bacteria belonging to a serovar and/or serogroup. Moreover, organisms which belong to different species may be classified into the same serovar or serogroup because they are indistinguishable by antigenic determination. As used herein, the term "serovar" means one or more *Lawsonia* strains which are antigenically-identical with respect to antigenic determinants produced by one or more loci. Quantitatively, serovars may be differentiated from one another by cross-agglutination absorption techniques. As used herein, the term "serogroup" refers to a group of *Lawsonia spp.* whose members cross-agglutinate with shared group antigens and do not cross-agglutinate with the members of other groups and, as a consequence, the members of a serogroup have more or less close antigenic relations with one another by simple cross-agglutination.



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The present invention thus clearly extends to vaccine compositions for the treatment and/or prophylaxis of animals, in particular, vaccine compositions for the treatment and/or prophylaxis of porcine and/or avian species, against any bacterium belonging to the same serovar or serogroup as *L. intracellularis*. Preferably, such organisms will express a polypeptide homologue, analogue or derivative of a polypeptide selected from the group consisting of flhB, flhR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN polypeptides.

The present invention extends further to vaccine compositions capable of conferring protection against a "genetic variant" of *L. intracellularis*, the only requirement being that said variant expresses a polypeptide which is immunologically cross-reactive to a polypeptide selected from the group consisting of flhB, flhR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN polypeptides. Genetic variants of *L. intracellularis* can be developed by mutation, recombination, conjugation or transformation of *L. intracellularis* or may occur naturally. It will be known to a person skilled in the art how to produce such derivatives.

In a particularly preferred embodiment, the vaccine composition of the invention is intended for or suitable for the prophylaxis and/or treatment of infection in a porcine or avian animal and more preferably, for prophylaxis and/or treatment of a porcine animal for infection by *L. intracellularis*.

Accordingly, the present invention clearly extends to the use of the immunogenic polypeptide of the invention or a DNA or RNA molecule encoding the same, according to any one of the preceding embodiments or as exemplified herein in the preparation of a medicament for the treatment and/or prophylaxis of PPE in animals, particularly porcine or avian animals.

The invention further extends to a method of treatment and/or prophylaxis of PPE in an animal such as an avian or porcine animal, said method comprising administering



the vaccine composition or the immunogenic polypeptide of the invention or a DNA or RNA molecule encoding the same, as described or exemplified herein to said animal for a time and under conditions sufficient for an immune response to occur thereto.

Preferably, in the case of administration of a vaccine composition, the immune  
5 response to the immunogen is a protective immune response.

Those skilled in the art will recognise the general applicability of the invention in vaccinating animals other than porcine and avian animals against *L. intracellularis* and/or related microorganisms. In the general application of the vaccine of the present  
10 invention, the only prerequisite is that the animal on which protection is conferred is capable of being infected with *L. intracellularis* and/or a related microorganism thereto and that, in the case of a related microorganism to *L. intracellularis*, said related microorganism expresses a B-cell or T-cell epitope which mimics or cross-reacts with the polypeptide component of the vaccine composition described herein. Animals  
15 which may be protected by the vaccine of the present invention include, but are not limited to, humans, primates, companion animals (e.g., cats, dogs), livestock animals (e.g., pigs, sheep, cattle, horses, donkeys, goats), laboratory test animals (e.g., mice, rats, guinea pigs, rabbits) and captive wild animals (e.g., kangaroos, foxes, deer). The present invention also extends to the vaccination of birds such as poultry birds, game  
20 birds and caged birds.

The present invention further extends to combination vaccines comprising an effective amount of a first immunogenic component comprising a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides,  
25 or a homologue, analogue or derivative thereof as described herein, or a DNA or RNA molecule encoding the same, combined with an effective amount of a second immunogenic component comprising one or more other antigens capable of protecting a porcine animal, or bird, against either *Lawsonia spp.* or another pathogen that infects and causes disease in said animal. The second immunogenic component is different  
30 from the first immunogenic component and is preferably selected from the group consisting of the *L. intracellularis* FlgE, hemolysin, OmpH, SodC, flhB, fliR, ntrC, glnH,



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motA, motB, tlyC, ytfM, and ytfN polypeptides and homologues, analogues or derivatives thereof. The present invention clearly extends to DNA vaccines and vaccine vectors which express said first immunogenic component and said second immunogenic component.

5

It is within the scope of the invention to encompass vaccine compositions comprising multimeric and polymeric forms of any one or more of the immunogenic polypeptides described herein, such as tandem arrays of homologous amino acid sequences, or, alternatively, tandem arrays of heterologous immunogenic repeats of amino acid sequences. The present invention extends further to nucleic acid molecules encoding such polymeric forms.

10

The isolated or recombinant polypeptide of the invention, or an immunologically-equivalent homologue, analogue or derivative thereof is also useful for the preparation of immunologically interactive molecules which are useful in the diagnosis of infection of an animal by *Lawsonia spp.*, in particular by *L. intracellularis* or a related organism thereto.

15

As used herein, the term "immunologically interactive molecule" includes antibodies and antibody derivatives and functional equivalents, such as a Fab, or a SCAB (single-chain antibody), any of which optionally can be conjugated to an enzyme, radioactive or fluorescent tag, amongst others. The only requirement of such immunologically interactive molecules is that they are capable of binding specifically to the immunogenic polypeptide of the present invention as hereinbefore described.

20

25

Accordingly, a further aspect of the invention extends to an immunologically interactive molecule which is capable of binding to a polypeptide selected from the group consisting of:

- (i) a polypeptide which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

30

(ii) a polypeptide which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH);  
 5 NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(iv) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos:  
 15 NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(vi) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to the nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and  
 25 NM00/16482 (plasmid pGTE#7 ytfM);

(vii) a polypeptide encoded by at least about 15 contiguous nucleotides of a

nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(viii) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(ix) a homologue, analogue or derivative of any one of (i) to (viii) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

In a preferred embodiment, the immunologically interactive molecule is an antibody that binds specifically to one or more epitopes of a polypeptide selected from the group consisting of flhB, flhR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides. More preferably, the immunologically interactive molecule binds specifically to one or more epitopes of a polypeptide from a causative agent of PPE, such as, for example, *L. intracellularis*.

Conventional methods can be used to prepare the immunologically interactive molecules. For example, by using a polypeptide immunogen of the present invention, polyclonal antisera or monoclonal antibodies can be made using standard methods. For example, a mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the polypeptide of the present invention which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a polypeptide include conjugation to carriers, or other techniques well known in the art. For example, the polypeptide can be administered in the presence of adjuvant or can be coupled to a carrier molecule, as known in the art, that enhances the immunogenicity of the polypeptide. The progress of immunization can be monitored by detection of antibody titres in plasma or serum. Standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of

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antibodies. Following immunization, antisera can be obtained and, for example, IgG molecules corresponding to the polyclonal antibodies can be isolated from the antisera.

5 To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an animal immunised with a polypeptide of the present invention and fused with myeloma cells by standard somatic cell fusion procedures, thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, for example, the hybridoma technique originally developed by Kohler  
10 and Milstein (1975), as well as other techniques such as the human B-cell hybridoma technique (Kozbor *et al.*, 1983), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, 1985), and screening of combinatorial antibody libraries (Huse *et al.*, 1989). Hybridoma cells can be isolated and screened immunochemically for production of antibodies that are specifically reactive with the  
15 polypeptide and monoclonal antibodies isolated therefrom.

As with all immunogenic compositions for eliciting antibodies, the immunogenically effective amounts of the peptides of the invention must be determined empirically. Factors to be considered include the immunogenicity of the native polypeptide,  
20 whether or not the polypeptide will be complexed with or covalently attached to an adjuvant or carrier protein or other carrier, the route of administration for the composition, i.e., intravenous, intramuscular, subcutaneous, *etc.*, and the number of immunizing doses to be administered. Such factors are known in the vaccine art and it is well within the skill of immunologists to make such determinations without undue  
25 experimentation.

The term "antibody" as used herein, is intended to include fragments thereof which are also specifically reactive with a polypeptide that mimics or cross-reacts with a B-cell or T-cell epitope of the *L. intracellularis* polypeptide selected from the group consisting  
30 of flhB, flhR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides. Antibodies can be fragmented using conventional techniques and the fragments screened for utility

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in the same manner as described above for whole antibodies. For example, F(ab')<sub>2</sub> fragments can be generated by treating antibody with pepsin. The resulting F(ab')<sub>2</sub> fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

- 5 It is within the scope of this invention to include any secondary antibodies (monoclonal, polyclonal or fragments of antibodies), including anti-idiotypic antibodies, directed to the first mentioned antibodies discussed above. Both the first and second antibodies can be used in detection assays or a first antibody can be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes
- 10 any antibody specific to any region of a polypeptide which mimics, or cross-reacts with a B-cell or T-cell epitope of a *L. intracellularis* polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides.

- The antibodies described herein are useful for determining B-cell or T-cell epitopes of
- 15 a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, such as, for example, by testing the ability of synthetic peptides to cross-react immunologically with said polypeptide or to elicit the production of antibodies which cross-react with said polypeptide. Using methods described herein, polyclonal antibodies, monoclonal antibodies or chimeric monoclonal
- 20 antibodies can also be raised to peptides which mimic or cross-react with a B-cell or T-cell epitope of a *L. intracellularis* polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides.

- More particularly, the polyclonal, monoclonal or chimeric monoclonal antibodies can
- 25 be used to detect the polypeptide of the invention and/or any homologues, analogues or derivatives thereof, in various biological materials. For example, they can be used in an ELISA, radioimmunoassay, or histochemical test. In other words, the antibodies can be used to test for binding to a polypeptide of the invention or to a homologue, analogue or derivative thereof, in a biological sample to diagnose the presence of *L.*
- 30 *intracellularis* therein.

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- Accordingly, a further aspect of the invention provides a method of diagnosing infection of an animal by *L. intracellularis* or a related microorganism thereto, said method comprising the steps of contacting a biological sample derived from said animal with an immunologically interactive molecule which is capable of binding to a polypeptide selected from the group consisting of flhB, flhR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue or derivative thereof, for a time and under conditions sufficient for an antigen:antibody complex to form, and detecting said complex formation.
- 10 According to this embodiment of the present invention, the immunologically interactive molecule is preferably an antibody molecule prepared against a *L. intracellularis* polypeptide selected from the group consisting of flhB, flhR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or an analogue or derivative thereof.
- 15 If the biological sample being tested contains one or more epitopes of a polypeptide selected from the group consisting of flhB, flhR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or an immunologically cross-reactive homologue, analogue or derivative thereof, it will give a positive binding result to the immunologically interactive molecule.
- 20 Preferably, the biological sample is derived from a porcine or avian host of the pathogen *L. intracellularis* or a related microorganism thereto, and includes an appropriate tissue or fluid sample from the animal.
- 25 Preferred biological samples are derived from the ileum, caecum, small intestine, large intestine, whole serum or lymph nodes of the porcine or avian host animal being tested. Alternatively or in addition the biological test sample may comprise faeces or a rectal swab derived from the animal.
- 30 To distinguish *L. intracellularis* from other microorganisms resident in the gut or other organ of an animal, the antibodies should not be prepared against highly-conserved

epitopes of the *L. intracellularis* polypeptide, such as, for example, those amino acid sequences of at least 5 amino acids in length which are conserved between *L. intracellularis* and a microorganism which is present in the gut or other organ of an animal in respect of which diagnosis is sought such as, for example, *E.coli*.

5

Conventional immunoassays can be used to perform this embodiment of the invention. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These, of course, include both single-site and two-site or "sandwich" assays of the non-competitive types, as well  
10 as the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target. It will be readily apparent to the skilled technician how to modify or optimise such assays to perform this embodiment of the present invention, and all such modifications and optimisations are encompassed by the present invention.

15

In one alternative embodiment, the present invention contemplates a method of identifying whether or not an animal has suffered from a past infection, or is currently infected with *L. intracellularis* or a related microorganism thereto, said method comprising contacting blood or serum derived from said animal with the immunogenic  
20 polypeptide of the invention for a time and under conditions sufficient for an antigen:antibody complex to form, and detecting said complex formation. This embodiment differs from the embodiment described *supra* in that it relies upon the detection of circulating antibodies against *L. intracellularis* or related organism in the animals blood or serum which are present as a consequence of a past or present  
25 infection by this pathogen. However, it will be apparent to those skilled in the art that the principle of the assay format is the same. As with other embodiments of the invention referred to *supra*, conventional immunoassays can be used. Persons skilled in the art will readily be capable of varying known immunoassay formats to perform the present embodiment. This embodiment of the invention can also utilise derivatives of  
30 blood and serum which comprise immunologically interactive molecules such as, for example, partially-purified IgG or IgM fractions and buffy coat samples, amongst

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others. The preparation of such fractions will also be known to those skilled in the art.

A further aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides that encodes, or is complementary to a nucleic acid molecule that encodes a polypeptide selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* polypeptides, including any and all genes selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes as defined hereinabove.

10 In a preferred embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide that is immunologically cross-reactive with *L. intracellularis* or other causative agent of PPE, wherein said nucleotide sequence is selected from the group consisting of:

- 15 (i) a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17 or a degenerate variant thereof;
- (ii) a nucleotide sequence having at least about 60% sequence identity overall to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 *glnH*); NM00/16477 (plasmid pGTE#2 *flhB*); NM00/16478 (plasmid pGTE#3 *fliR*); NM00/16479 (plasmid pGTE#4 *motA/B*); NM00/16480 (plasmid pGTE#5 *tlyC*); NM00/16481 (plasmid pGTE#6 *ntrC*); and NM00/16482 (plasmid pGTE#7 *ytfM*); or a degenerate variant thereof;
- 20 (iii) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;
- 25 (iv) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 *glnH*); NM00/16477 (plasmid pGTE#2 *flhB*); NM00/16478 (plasmid pGTE#3 *fliR*); NM00/16479 (plasmid pGTE#4 *motA/B*); NM00/16480 (plasmid pGTE#5
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tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) a nucleotide sequence which hybridizes under at least low stringency conditions to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17 or a complementary nucleotide sequence thereto;

(vi) a nucleotide sequence which hybridizes under at least low stringency conditions to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(vii) a homologue, analogue or derivative of any one of (i) to (vi) which encodes a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

For the present purpose, a "homologue" of a nucleotide sequence shall be taken to refer to an isolated nucleic acid molecule which encodes a polypeptide that is immunologically cross-reactive to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, but which includes one or more nucleotide substitutions, insertions, deletions, or rearrangements.

An "analogue" of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which encodes a polypeptide which is immunologically cross-reactive to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, but which includes one or more non-nucleotide constituents not normally present in said isolated nucleic acid molecule, such as, for example, carbohydrates, radiochemicals including radio nucleotides, reporter molecules such as, but not limited to biotin, DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

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A "derivative" of a nucleotide sequence set forth herein shall be taken to refer to any isolated nucleic acid molecule which contains at least about 60% nucleotide sequence identity to 15 or more contiguous nucleotides present in the nucleotide sequence of a gene selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes.

Generally, a *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, or *ytfN* gene may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues. Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence of the gene, although random insertion is also possible with suitable screening of the resulting product being performed. Deletional nucleotide sequence variants are characterised by the removal of one or more nucleotides from the gene. Substitutional nucleotide sequence variants are those in which at least one nucleotide in the gene sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place. In a preferred embodiment, such substitutions are selected based on the degeneracy of the genetic code, as known in the art, with the resulting substitutional variant encoding the amino acid sequence of a *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, or *ytfN* polypeptide.

Preferred homologues, analogues and derivatives of a *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, or *ytfN* gene comprise a sequence of nucleotides which has at least about 80% identity, even more preferably at least about 90% identity, and yet still more preferably at least about 95% identity to said gene.

In determining whether or not two nucleotide sequences fall within these percentage limits, reference is made to the description *supra* of methods for conducting a side-by-side comparison or multiple alignment of nucleotide sequences.

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Alternatively or in addition, preferred homologues, analogues and derivatives of a *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, or *ytfN* gene comprise a sequence of nucleotides which hybridizes under at least moderate stringency conditions and to the nucleotide sequence of said gene, or to a nucleic acid fragment comprising at least  
 5 about 20 contiguous nucleotides in length derived therefrom, and even more preferably, under high stringency conditions to said gene, or to said nucleic acid fragment. For the purposes of defining the level of stringency, reference is made to the description hereinabove of hybridization stringencies.

10 In a more preferred embodiment, such a nucleotide sequence encodes a polypeptide that is immunologically cross-reactive with *L. intracellularis* or other causative agent of PPE.

In a particularly preferred embodiment, the isolated nucleic acid molecule of the  
 15 present invention comprises or consists of a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1,3, 5, 7, 9, 11, 13, 15, and 17;
- (ii) a nucleotide sequence of the *L. intracellularis* DNA contained within a  
 20 deposited plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 *glnH*); NM00/16477 (plasmid pGTE#2 *flhB*); NM00/16478 (plasmid pGTE#3 *fliR*); NM00/16479 (plasmid pGTE#4 *motA/B*); NM00/16480 (plasmid pGTE#5 *tlyC*); NM00/16481 (plasmid pGTE#6 *ntrC*); and NM00/16482 (plasmid pGTE#7 *ytfM*); and
- 25 (iii) a nucleotide sequence that encodes the same polypeptide as (i) or (ii), wherein said polypeptide is selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* polypeptides; and
- (iv) a nucleotide sequence that is complementary to (i) or (ii) or (iii).

30 The present invention clearly encompasses genetic constructs comprising the subject nucleic acid molecule in an expressible format suitable for the preparation of a

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recombinant immunogenic polypeptide selected from the group consisting of flhB, flhR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, such as for use in recombinant univalent or polyvalent recombinant vaccines.

- 5 In such cases, the nucleic acid molecule will be operably connected to a promoter sequence which can thereby regulate expression of said nucleic acid molecule in a prokaryotic or eukaryotic cell as described *supra*.

10 The genetic construct optionally further comprises a terminator sequence. The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. A "terminator" is a nucleotide sequence, generally located within the 3'-non-translated region of a gene or mRNA, comprising a polyadenylation signal to facilitate the post-transcriptional addition of a polyadenylate sequence to the 3'-end of a primary mRNA transcript. Terminator sequences may be isolated from the  
15 genetic sequences of bacteria, fungi, viruses, animals and/or plants. Terminators active in animal cells are known and described in the literature.

In a preferred embodiment, the genetic construct can be a cloning or expression vector, as known in the art, such as a plasmid, cosmid, or phage, comprising a nucleic  
20 acid molecule of the present invention, and host cells transformed or transfected therewith. In a non-limiting embodiment, the vector is a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC);  
25 NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

The genetic constructs of the present invention are particularly useful for producing the immunogenic component of the vaccine composition described herein or for use in a  
30 DNA vaccine.

A range of genetic diagnostic assays to detect infection of an animal by *L.*

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*intracellularis* or a related microorganism can be employed using the nucleic acid molecule described herein such as, for example, assays based upon the polymerase chain reaction (PCR) and nucleic acid hybridisation. All such assays are contemplated in the present invention.

5

Accordingly, a still further aspect of the invention provides a diagnostic method of detecting *L. intracellularis* or related microorganism in a biological sample derived from an animal subject, said method comprising the steps of hybridising one or more probes or primers derived from a nucleotide sequence of a *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*,  
10 *tlyC*, *ytfM*, or *ytfN* gene as defined hereinabove, or a homologue, analogue or derivative thereof, to a DNA or RNA molecule present in said sample and then detecting said hybridisation using a detection means.

As used herein, the term "probe" refers to a nucleic acid molecule which is capable of  
15 being used in the detection of a gene selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes. Probes may comprise DNA (single-stranded or double-stranded) or RNA (i.e., riboprobes) or analogues thereof.

The term "primer" refers to a probe as hereinbefore defined which is further capable  
20 of being used to amplify a nucleotide sequence from *L. intracellularis* or a related microorganism thereto in a PCR.

Preferred probes and primers include fragments of a gene selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes, including  
25 synthetic single-stranded DNA or RNA molecules of at least about 15 nucleotides in length.

Preferably, probes and primers according to this embodiment will comprise at least about 20 contiguous nucleotides in length from a gene selected from the group  
30 consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes, even more preferably at least about 25 contiguous nucleotides, still even more preferably at least

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about 50 contiguous nucleotides, and even more preferably at least about 100 nucleotides to about 500 nucleotides in length from said gene. Probes and primers comprising the full-length gene or a complementary nucleotide sequence thereto are also encompassed by the present invention.

5

Probes or primers can comprise inosine, adenine, guanine, thymidine, cytidine or uracil residues or functional analogues or derivatives thereof that are capable of being incorporated into a polynucleotide molecule, provided that the resulting probe or primer is capable of hybridising under at least low stringency conditions to a gene selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes, or is at least about 60% identical to one strand of said gene.

10

The biological sample according to this aspect of the invention includes any organ, tissue, cell or exudate which contains or is likely to contain *L. intracellularis* or a nucleic acid derived therefrom. A biological sample can be prepared in a suitable solution such as, for example, an extraction buffer or suspension buffer. The present invention extends to the testing of biological solutions thus prepared, the only requirement being that said solution at least comprises a biological sample as described herein.

15

The diagnostic assay of the present invention is useful for the detection of *L. intracellularis* or a microorganism which is related thereto which expresses a polypeptide selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* polypeptides.

20

The present invention clearly contemplates diagnostic assays which are capable of both genus-specific and species-specific detection. Accordingly, in one embodiment, the probe or primer, or a homologue, analogue or derivative thereof, comprises DNA capable of being used to detect multiple *Lawsonia spp.* In an alternative embodiment, the probe or primer or a homologue, analogue or derivative thereof comprises DNA capable of being used to distinguish *L. intracellularis* from related microorganisms.

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Less-highly conserved regions within the *flhB*, *fliR*, *ntnC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, or *ytfN* genes are particularly useful as species-specific probes and/or primers for the detection of *L. intracellularis* and very closely related species.

5 Furthermore, the diagnostic assays described herein can be adapted to a genus-specific or species-specific assay by varying the stringency of the hybridisation step. Accordingly, a low stringency hybridisation can be used to detect several different species of *Lawsonia* in one or more biological samples being assayed, while a high stringency hybridisation can be used to distinguish *L. intracellularis* from such other  
10 species.

The detection means according to this aspect of the invention may be any nucleic acid-based detection means such as, for example, nucleic acid hybridisation techniques or paper chromatography hybridisation assay (PACHA), or an amplification reaction such  
15 as PCR, or nucleic acid sequence-based amplification (NASBA) system. The invention further encompasses the use of different assay formats of said nucleic acid-based detection means, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), single-strand chain polymorphism (SSCP), amplification and mismatch detection (AMD), interspersed repetitive sequence  
20 polymerase chain reaction (IRS-PCR), inverse polymerase chain reaction (iPCR), *in situ* polymerase chain reaction and reverse transcription polymerase chain reaction (RT-PCR), amongst others.

Where the detection means is a nucleic acid hybridisation technique, the probe can  
25 be labelled with a reporter molecule capable of producing an identifiable signal (e.g., a radioisotope such as <sup>32</sup>P or <sup>35</sup>S, or a biotinylated molecule). According to this embodiment, those skilled in the art will be aware that the detection of said reporter molecule provides for identification of the probe and that, following the hybridisation reaction, the detection of the corresponding nucleotide sequences in the biological  
30 sample is facilitated. Additional probes can be used to confirm the assay results obtained using a single probe.

A variation of the nucleic acid hybridisation technique contemplated by the present invention is the paper chromatography hybridisation assay (PACHA) described by Reinhartz *et al.* (1993) and equivalents thereof, wherein a target nucleic acid molecule is labelled with a reporter molecule such as biotin, applied to one end of a nitrocellulose or nylon membrane filter strip and subjected to chromatography under the action of capillary or other forces (e.g., an electric field) for a time and under conditions sufficient to promote migration of said target nucleic acid along the length of said membrane to a zone at which a DNA probe is immobilised thereto such as, for example, in the middle region. According to this detection format, labelled target nucleic acid comprising the *Lawsonia spp.* nucleotide sequences complementary to the probe will hybridise thereto and become immobilised in that region of the membrane to which the probe is bound. Non-complementary sequences to the probe will diffuse past the site at which the probe is bound. The target nucleic acid may comprise a crude or partially-pure extract of DNA or RNA or, alternatively, an amplified or purified DNA. Additional variations of this detection means which utilise the nucleotide sequences described herein are clearly encompassed by the present invention.

Wherein the detection means is a RFLP, nucleic acid derived from the biological sample, in particular DNA, is digested with one or more restriction endonuclease enzymes and the digested DNA is subjected to electrophoresis, transferred to a solid support such as, for example, a nylon or nitrocellulose membrane, and hybridised to a probe optionally labelled with a reporter molecule as hereinbefore defined. According to this embodiment, a specific pattern of DNA fragments is displayed on the support, wherein said pattern is preferably specific for a particular *Lawsonia spp.*, to enable the user to distinguish between different species of the bacterium.

Wherein the detection means is an amplification reaction such as, for example, a polymerase chain reaction or a nucleic acid sequence-based amplification (NASBA) system or a variant thereof, one or more nucleic acid primer molecules of at least 15

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contiguous nucleotides in length derivable from a gene selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes is hybridised to nucleic acid derived from a biological sample, and nucleic acid copies of the FlgE-encoding genetic sequences in said sample, or a part or fragment thereof, are enzymically-amplified.

Those skilled in the art will be aware that there must be a sufficiently high percentage of nucleotide sequence identity between the primers and the sequences in the biological sample template molecule to which they hybridise (i.e., the "template molecule"). As stated previously, the stringency conditions can be selected to promote hybridisation.

Preferably, each primer is at least about 95% identical to a region of a gene selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes in the template molecule to which it hybridises.

Those skilled in the art will also be aware that, in one format, PCR provides for the hybridisation of non-complementary primers to different strands of the template molecule, such that the hybridised primers are positioned to facilitate the 5'→ 3' synthesis of nucleic acid in the intervening region, under the control of a thermostable DNA polymerase enzyme. As a consequence, PCR provides an advantage over other detection means in so far as the nucleotide sequence in the region between the hybridised primers may be unknown and unrelated to any known nucleotide sequence.

In an alternative embodiment, wherein the detection means is AFLP, the primers are selected such that, when nucleic acid derived from the biological sample, in particular DNA, is amplified, different length amplification products are produced from different *Lawsonia spp.* The amplification products can be subjected to electrophoresis, transferred to a solid support such as, for example, a nylon or nitrocellulose membrane, and hybridised to a probe optionally labelled with a reporter molecule as hereinbefore described. According to this embodiment, a specific pattern of amplified

DNA fragments is displayed on the support, said pattern optionally specific for a particular *Lawsonia* spp., to enable the user to distinguish between different species of the bacterium in much the same way as for RFLP analysis.

- 5 The technique of AMD facilitates, not only the detection of *Lawsonia* spp. DNA in a biological sample, but also the determination of nucleotide sequence variants which differ from the primers and probes used in the assay format. Wherein the detection means is AMD, the probe is end-labelled with a suitable reporter molecule and mixed with an excess of the amplified template molecule. The mixtures are subsequently
- 10 denatured and allowed to renature to form nucleic acid "probe:template hybrid molecules" or "hybrids", such that any nucleotide sequence variation between the probe and the template molecule to which it is hybridised will disrupt base-pairing in the hybrids. These regions of mismatch are sensitive to specific chemical modification using hydroxylamine (mismatched cytosine residues) or osmium tetroxide (mismatched
- 15 thymidine residues), allowing subsequent cleavage of the modified site using piperidine. The cleaved nucleic acid may be analysed using denaturing polyacrylamide gel electrophoresis, followed by standard nucleic acid hybridisation as described *supra*, to detect the *Lawsonia*-derived nucleotide sequences. Those skilled in the art will be aware of the means of end-labelling a genetic probe according to the performance of
- 20 the invention described in this embodiment.

According to this embodiment, the use of a single end-labelled probe allows unequivocal localisation of the sequence variation. The distance between the point(s) of sequence variation and the end-label is represented by the size of the cleavage

25 product.

In an alternative embodiment of AMD, the probe is labelled at both ends with a reporter molecule, to facilitate the simultaneous analysis of both DNA strands.

- 30 Wherein the detection means is RT-PCR, the nucleic acid sample comprises an RNA molecule which is a transcription product of *Lawsonia*-derived DNA or a homologue,

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analogue or derivative thereof. As a consequence, this assay format is particularly useful when it is desirable to determine expression of one or more *Lawsonia* genes.

According to this embodiment, the RNA sample is reverse-transcribed to produce the complementary single-stranded DNA which is subsequently amplified using standard  
5 procedures.

Variations of the embodiments described herein are described in detail by McPherson *et al.* (1991).

10 The present invention clearly extends to the use of any and all detection means referred to *supra* for the purposes of diagnosing *Lawsonia spp.* and in particular *L. intracellularis* infection in animals.

The amplification reaction detection means described *supra* can be further coupled to  
15 a classical hybridisation reaction detection means to further enhance sensitivity and specificity of the inventive method, such as by hybridising the amplified DNA with a probe which is different from any of the primers used in the amplification reaction.

Similarly, the hybridisation reaction detection means described *supra* can be further  
20 coupled to a second hybridisation step employing a probe which is different from the probe used in the first hybridisation reaction.

A further aspect of the invention provides an isolated probe or primer derived from a gene selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*,  
25 *ytfM*, and *ytfN* genes. Preferably, the probe or primer of the invention comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 19 to SEQ ID NO: 46 or a complementary nucleotide sequence thereto.

The present invention does not extend to any nucleic acid or polypeptide of  
30 *Campylobacter* or *Helicobacter* that was disclosed publicly before the filing date or priority date of this application, or otherwise takes priority over the instant application,

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and which is homologous to a nucleotide sequence or amino acid sequence of *Lawsonia spp.* disclosed herein.

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## SEQUENCE LISTING

&lt;110&gt; US ONLY:

Robert T. GOOD; Richard A. STRUGNELL; Everett L. ROSEY; and Kendall W. KING

5

OTHER COUNTRIES:

Agriculture Victoria Services Pty Ltd AND Pig Research and Development Corporation AND Pfizer Products Inc.

10 <120> Novel therapeutic compositions for treating infection by *Lawsonia spp.*

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25 gcg cat cct ttc gat cta gta ttg tta atc ata agc gag gtt ttt ctt 240  
 Ala His Pro Phe Asp Leu Val Leu Leu Ile Ile Ser Glu Val Phe Leu  
 65 70 75 80

30 ggt att gta ttg ggg ctt gcg gta aac ttt ttc ttt gca gga att caa 288  
 Gly Ile Val Leu Gly Leu Ala Val Asn Phe Phe Phe Ala Gly Ile Gln  
 85 90 95

35 gct ggg gga gaa att ctt gct aca caa atg ggg ttt aca atg att acg 336  
 Ala Gly Gly Glu Ile Leu Ala Thr Gln Met Gly Phe Thr Met Ile Thr  
 100 105 110

40 ctt gca gac cca tta act ggt aac acc aca ggt ttt att gca cat ttt 384  
 Leu Ala Asp Pro Leu Thr Gly Asn Thr Thr Gly Phe Ile Ala His Phe  
 115 120 125

45 ctt tat atg gtt gct aca tta gtt ttt ctt gct ctt aat ggc cat ttg 432  
 Leu Tyr Met Val Ala Thr Leu Val Phe Leu Ala Leu Asn Gly His Leu  
 130 135 140

ttt ctt ata aaa gct ttt aca tat act ttt aaa atg gtt cca gca gga 480  
 Phe Leu Ile Lys Ala Phe Thr Tyr Thr Phe Lys Met Val Pro Ala Gly  
 145 150 155 160

- 79 -

gga ctt gtt gta aga gaa att tta ttg agt gaa ctt ctt aat atg gca 528  
 Gly Leu Val Val Arg Glu Ile Leu Leu Ser Glu Leu Leu Asn Met Ala  
                     165                    170                    175

5 ggg atg att ttt gtt ttt gcc tta cat gtt gcg gca cca gtt atg tca 576  
 Gly Met Ile Phe Val Phe Ala Leu His Val Ala Ala Pro Val Met Ser  
                     180                    185                    190

10 gct ctt ttt tta gta gag atc tct tta gga ctt atg gca aga gct gct 624  
 Ala Leu Phe Leu Val Glu Ile Ser Leu Gly Leu Met Ala Arg Ala Ala  
                     195                    200                    205

15 cct cag att cat att atg gaa gtt gga ttt cct gta aaa att ggt gta 672  
 Pro Gln Ile His Ile Met Glu Val Gly Phe Pro Val Lys Ile Gly Val  
                     210                    215                    220

20 gga ttt ttt ttc att gga cta tta ttt act atc tta tca aaa gaa acc 720  
 Gly Phe Phe Phe Ile Gly Leu Leu Phe Thr Ile Leu Ser Lys Glu Thr  
                     225                    230                    235                    240

tat cga ttt att gca ggc cta gag gga cta ttt ttt aac tta ctt act 768  
 Tyr Arg Phe Ile Ala Gly Leu Glu Gly Leu Phe Phe Asn Leu Leu Thr  
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25 gta atg ggt agt gga aaa tag 789  
 Val Met Gly Ser Gly Lys  
                     260

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                     20                    25                    30

Ile Asp Gly Phe Pro Asn Met Leu Lys Ala Ser Ile Ala Leu Ile Leu  
                     35                    40                    45

45 Thr Ile Val Leu Trp Gly Arg Leu Ser Leu Ser Gly Thr Gln Met Pro

- 80 -

	50		55		60
	Ala His Pro Phe Asp Leu Val Leu Leu Ile Ile Ser Glu Val Phe Leu				
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5	Gly Ile Val Leu Gly Leu Ala Val Asn Phe Phe Phe Ala Gly Ile Gln				
		85		90	95
	Ala Gly Gly Glu Ile Leu Ala Thr Gln Met Gly Phe Thr Met Ile Thr				
10		100		105	110
	Leu Ala Asp Pro Leu Thr Gly Asn Thr Thr Gly Phe Ile Ala His Phe				
	115		120		125
15	Leu Tyr Met Val Ala Thr Leu Val Phe Leu Ala Leu Asn Gly His Leu				
	130		135		140
	Phe Leu Ile Lys Ala Phe Thr Tyr Thr Phe Lys Met Val Pro Ala Gly				
20	145		150		155 160
	Gly Leu Val Val Arg Glu Ile Leu Leu Ser Glu Leu Leu Asn Met Ala				
		165		170	175
	Gly Met Ile Phe Val Phe Ala Leu His Val Ala Ala Pro Val Met Ser				
25		180		185	190
	Ala Leu Phe Leu Val Glu Ile Ser Leu Gly Leu Met Ala Arg Ala Ala				
	195		200		205
30	Pro Gln Ile His Ile Met Glu Val Gly Phe Pro Val Lys Ile Gly Val				
	210		215		220
	Gly Phe Phe Phe Ile Gly Leu Leu Phe Thr Ile Leu Ser Lys Glu Thr				
35	225		230		235 240
	Tyr Arg Phe Ile Ala Gly Leu Glu Gly Leu Phe Phe Asn Leu Leu Thr				
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<213> Lawsonia intracellularis

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ca ttg aaa gga att ttt gaa gat gag ggc cat gaa gtt tta gaa aga 96  
Ser Leu Lys Gly Ile Phe Glu Asp Glu Gly His Glu Val Leu Glu Arg  
20 25 30

15 gct tca gca gaa gaa gga ctt aag tgt gtt gat gta gag tct cca gat 144  
Ala Ser Ala Glu Glu Gly Leu Lys Cys Val Asp Val Glu Ser Pro Asp  
35 40 45

20    ctt gtt ttt ctt gat att tgg ctt cct ggg atg gat ggt ctt atg gct    192  
       Leu Val Phe Leu Asp Ile Trp Leu Pro Gly Met Asp Gly Leu Met Ala  
           50                    55                    60

25      tta gac cat att cag gct ctt cat cag gaa tta cct gtt att atg att      240  
Leu Asp His Ile Gln Ala Leu His Gln Glu Leu Pro Val Ile Met Ile  
65                      70                      75                      80

tca ggt cat gcc aca att gaa act gct gta aca gct atc cgt caa ggt 288  
 Ser Gly His Ala Thr Ile Glu Thr Ala Val Thr Ala Ile Arg Gln Gly  
 30 85 90 95

gct tat gat ttt att gaa aag cct ctt tct ttg gaa aaa gtc ctt att 336  
Ala Tyr Asp Phe Ile Glu Lys Pro Leu Ser Leu Glu Lys Val Leu Ile  
100 105 110

aca gct aat aga gct ata gaa aca gta aga tta aga agg gaa aac aaa 384  
Thr Ala Asn Arg Ala Ile Glu Thr Val Arg Leu Arg Arg Glu Asn Lys  
115 120 125

40    tta cta cgt act gta tta cct gag gag agt gag ttt ata gga cag tct    432  
       Leu Leu Arg Thr Val Leu Pro Glu Glu Ser Glu Phe Ile Gly Gln Ser  
       130                    135                    140

45      cct gtt atc tta aaa ttt aaa agt tta tta tca cag gtc gct cca aca      480  
Pro Val Ile Leu Lys Phe Lys Ser Leu Leu Ser Gln Val Ala Pro Thr

- 82 -

	145	150	155	160	
	gat gct tgg gta cta ctt aca gga gag aat ggt aca ggt aaa gag tta				528
5	Asp Ala Trp Val Leu Leu Thr Gly Glu Asn Gly Thr Gly Lys Glu Leu				
	165		170	175	
	gct gca caa gca ttg cac aaa gga agc tca cga tat caa aaa cca ttt				576
	Ala Ala Gln Ala Leu His Lys Gly Ser Ser Arg Tyr Gln Lys Pro Phe				
10	180		185	190	
	ata gct gtt aat tgt gct gct atc cct gaa gaa ttg att gaa agc gaa				624
	Ile Ala Val Asn Cys Ala Ala Ile Pro Glu Glu Leu Ile Glu Ser Glu				
	195		200	205	
15	cta ttt ggt cat gaa aaa ggg gcc ttt act ggt gcc gat gct tct cgt				672
	Leu Phe Gly His Glu Lys Gly Ala Phe Thr Gly Ala Asp Ala Ser Arg				
	210		215	220	
	gca ggt cgt ttt gag ttg gca cat aaa gga aca tta ttt ctt gat gaa				720
20	Ala Gly Arg Phe Glu Leu Ala His Lys Gly Thr Leu Phe Leu Asp Glu				
	225		230	235	240
	ata gga gat atg agt tta aaa aca caa gca aaa att ttg cgt att ttg				768
25	Ile Gly Asp Met Ser Leu Lys Thr Gln Ala Lys Ile Leu Arg Ile Leu				
	245		250	255	
	caa gaa caa tgt ttt gaa aaa att ggt agt gtt aga act att aaa gtt				816
	Gln Glu Gln Cys Phe Glu Lys Ile Gly Ser Val Arg Thr Ile Lys Val				
30	260		265	270	
	gat gta aga gtt att gca gca aca aat aag aat ctt gaa gac gct att				864
	Asp Val Arg Val Ile Ala Ala Thr Asn Lys Asn Leu Glu Asp Ala Ile				
	275		280	285	
35	agc gat gga aca ttt cgt caa gat ttg tat tat cgc tta cga gtt gtt				912
	Ser Asp Gly Thr Phe Arg Gln Asp Leu Tyr Tyr Arg Leu Arg Val Val				
	290		295	300	
	cca ttg cat ctt ccc cct ctt cgt gaa cgt gat tct gat att gag cta				960
40	Pro Leu His Leu Pro Pro Leu Arg Glu Arg Asp Ser Asp Ile Glu Leu				
	305		310	315	320
	tta tta aat agg ttt gtg att cag ttg agt aaa cgt tat aga cgt gag				1008
45	Leu Leu Asn Arg Phe Val Ile Gln Leu Ser Lys Arg Tyr Arg Arg Glu				
	325		330	335	

- 83 -

ccg cct att ttt tta gat gag gtc ttc cct gta ttg aaa caa tat tgt 1056  
 Pro Pro Ile Phe Leu Asp Glu Val Phe Pro Val Leu Lys Gln Tyr Cys  
 340 345 350

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tgg cca ggg aat gta aga gaa tta ctt aat ttt gta gaa cga atg gtt 1104  
 Trp Pro Gly Asn Val Arg Glu Leu Leu Asn Phe Val Glu Arg Met Val  
 355 360 365

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att ctt tat tca ggg aag aaa gta tgt ttg aca gat cct aag gta aaa 1152  
 Ile Leu Tyr Ser Gly Lys Lys Val Cys Leu Thr Asp Pro Lys Val Lys  
 370 375 380

15

agc aat tta aaa tat tta ccc aag aaa ttt tct tcc cat tat aac ttt 1200  
 Ser Asn Leu Lys Tyr Leu Pro Lys Lys Phe Ser Ser His Tyr Asn Phe  
 385 390 395 400

20

ctt ccc gat ata gat ttt aac cag gct aaa ata gct ttt gaa cca aaa 1248  
 Leu Pro Asp Ile Asp Phe Asn Gln Ala Lys Ile Ala Phe Glu Pro Lys  
 405 410 415

25

ttt tta act gaa aaa tta cat gct tat caa gga aat att acc cga tta 1296  
 Phe Leu Thr Glu Lys Leu His Ala Tyr Gln Gly Asn Ile Thr Arg Leu  
 420 425 430

30

gca gaa gct att gga ctt gaa aga agt tat tta tat aga aag cta aaa 1344  
 Ala Glu Ala Ile Gly Leu Glu Arg Ser Tyr Leu Tyr Arg Lys Leu Lys  
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agc tat ggt att tat ctg tct gag tga 1371  
 Ser Tyr Gly Ile Tyr Leu Ser Glu  
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Met Ser Ala Arg Ile Leu Ile Ile Asp Asp Glu Asp Ser Ile Arg Phe  
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 Ser Leu Lys Gly Ile Phe Glu Asp Glu Gly His Glu Val Leu Glu Arg  
 20 25 30

- 84 -

Ala Ser Ala Glu Glu Gly Leu Lys Cys Val Asp Val Glu Ser Pro Asp  
35 40 45

5 Leu Val Phe Leu Asp Ile Trp Leu Pro Gly Met Asp Gly Leu Met Ala  
50 55 60

Leu Asp His Ile Gln Ala Leu His Gln Glu Leu Pro Val Ile Met Ile  
65 70 75 80

10 Ser Gly His Ala Thr Ile Glu Thr Ala Val Thr Ala Ile Arg Gln Gly  
85 90 95

Ala Tyr Asp Phe Ile Glu Lys Pro Leu Ser Leu Glu Lys Val Leu Ile  
15 100 105 110

Thr Ala Asn Arg Ala Ile Glu Thr Val Arg Leu Arg Arg Glu Asn Lys  
115 120 125

20 Leu Leu Arg Thr Val Leu Pro Glu Glu Ser Glu Phe Ile Gly Gln Ser  
130 135 140

Pro Val Ile Leu Lys Phe Lys Ser Leu Leu Ser Gln Val Ala Pro Thr  
145 150 155 160

25 Asp Ala Trp Val Leu Leu Thr Gly Glu Asn Gly Thr Gly Lys Glu Leu  
165 170 175

Ala Ala Gln Ala Leu His Lys Gly Ser Ser Arg Tyr Gln Lys Pro Phe  
30 180 185 190

Ile Ala Val Asn Cys Ala Ala Ile Pro Glu Glu Leu Ile Glu Ser Glu  
195 200 205

35 Leu Phe Gly His Glu Lys Gly Ala Phe Thr Gly Ala Asp Ala Ser Arg  
210 215 220

Ala Gly Arg Phe Glu Leu Ala His Lys Gly Thr Leu Phe Leu Asp Glu  
225 230 235 240

40 Ile Gly Asp Met Ser Leu Lys Thr Gln Ala Lys Ile Leu Arg Ile Leu  
245 250 255

Gln Glu Gln Cys Phe Glu Lys Ile Gly Ser Val Arg Thr Ile Lys Val  
45 260 265 270

- 85 -

Asp Val Arg Val Ile Ala Ala Thr Asn Lys Asn Leu Glu Asp Ala Ile  
 275 280 285

5 Ser Asp Gly Thr Phe Arg Gln Asp Leu Tyr Tyr Arg Leu Arg Val Val  
 290 295 300

Pro Leu His Leu Pro Pro Leu Arg Glu Arg Asp Ser Asp Ile Glu Leu  
 305 310 315 320

10 Leu Leu Asn Arg Phe Val Ile Gln Leu Ser Lys Arg Tyr Arg Arg Glu  
 325 330 335

Pro Pro Ile Phe Leu Asp Glu Val Phe Pro Val Leu Lys Gln Tyr Cys  
 15 340 345 350

Trp Pro Gly Asn Val Arg Glu Leu Leu Asn Phe Val Glu Arg Met Val  
 355 360 365

20 Ile Leu Tyr Ser Gly Lys Lys Val Cys Leu Thr Asp Pro Lys Val Lys  
 370 375 380

Ser Asn Leu Lys Tyr Leu Pro Lys Lys Phe Ser Ser His Tyr Asn Phe  
 385 390 395 400

25 Leu Pro Asp Ile Asp Phe Asn Gln Ala Lys Ile Ala Phe Glu Pro Lys  
 405 410 415

Phe Leu Thr Glu Lys Leu His Ala Tyr Gln Gly Asn Ile Thr Arg Leu  
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Ala Glu Ala Ile Gly Leu Glu Arg Ser Tyr Leu Tyr Arg Lys Leu Lys  
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35 Ser Tyr Gly Ile Tyr Leu Ser Glu  
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- 86 -

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 5        1                        5                        10                        15  
  
 aat ctt caa gtc aat ttt tct aac cca tac cat caa aca gat att gaa 96  
 Asn Leu Gln Val Asn Phe Ser Asn Pro Tyr His Gln Thr Asp Ile Glu  
                      20                        25                        30  
 10  
 gtc ctg gct aat gca aaa aaa gtt aaa ggg atg aag ttt cca caa gac 144  
 Val Leu Ala Asn Ala Lys Lys Val Lys Gly Met Lys Phe Pro Gln Asp  
                      35                        40                        45  
  
 ttt aat aaa cct gaa gtt ata gtt gct ata cgt aat ggt agt aca gtt 192  
 Phe Asn Lys Pro Glu Val Ile Val Ala Ile Arg Asn Gly Ser Thr Val  
                      50                        55                        60  
  
 att act cct gca aag caa ctt ctt cct aaa gca tct ttt aga ctc ttt 240  
 20    Ile Thr Pro Ala Lys Gln Leu Leu Pro Lys Ala Ser Phe Arg Leu Phe  
                      65                        70                        75                        80  
  
 gat gat gaa gtt gca tct ata aaa gat gta gaa tct gga caa tca cat 288  
 25    Asp Asp Glu Val Ala Ser Ile Lys Asp Val Glu Ser Gly Gln Ser His  
                      85                        90                        95  
  
 ata tta tta gct tca gca cca tta cca gcg att caa gct ata aac tca 336  
 Ile Leu Leu Ala Ser Ala Pro Leu Pro Ala Ile Gln Ala Ile Asn Ser  
                      100                        105                        110  
 30  
 aat ggc aac ctt att cgt tta gat aca ctc ccc att act cat caa tct 384  
 Asn Gly Asn Leu Ile Arg Leu Asp Thr Leu Pro Ile Thr His Gln Ser  
                      115                        120                        125  
  
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                      130                        135  
  
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 45    <400> 8

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Lys Gln Ile Asp Ile Ile Ile Val Gly Ala Thr Ile Thr Leu Glu Arg  
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 Asn Leu Gln Val Asn Phe Ser Asn Pro Tyr His Gln Thr Asp Ile Glu  
 5 20 25 30  
 Val Leu Ala Asn Ala Lys Lys Val Lys Gly Met Lys Phe Pro Gln Asp  
 35 40 45  
 10 Phe Asn Lys Pro Glu Val Ile Val Ala Ile Arg Asn Gly Ser Thr Val  
 50 55 60  
 Ile Thr Pro Ala Lys Gln Leu Leu Pro Lys Ala Ser Phe Arg Leu Phe  
 65 70 75 80  
 15 Asp Asp Glu Val Ala Ser Ile Lys Asp Val Glu Ser Gly Gln Ser His  
 85 90 95  
 Ile Leu Leu Ala Ser Ala Pro Leu Pro Ala Ile Gln Ala Ile Asn Ser  
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 ggc tac ctt atg gct aaa ggg aat ctt gct tta ctc ttt caa cct gca 96  
 Gly Tyr Leu Met Ala Lys Gly Asn Leu Ala Leu Leu Phe Gln Pro Ala  
 45 20 25 30

- 88 -

gaa ctt gtt atc att att ggg gca gca tta ggt gct ttt ttt gct tca 144  
 Glu Leu Val Ile Ile Ile Gly Ala Ala Leu Gly Ala Phe Phe Ala Ser  
 35 40 45

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cag acg aaa tat tca ttt act ctg gtc att aaa aat tta tca cac att 192  
 Gln Thr Lys Tyr Ser Phe Thr Leu Val Ile Lys Asn Leu Ser His Ile  
 50 55 60

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ttt ggc gat cca aac agt aca aaa ata aaa tac ctt gaa aca ctt gcc 240  
 Phe Gly Asp Pro Asn Ser Thr Lys Ile Lys Tyr Leu Glu Thr Leu Ala  
 65 70 75 80

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ctt ctc tat gga ctt ttc tta aaa atg aat aga gaa ggt gtc att agt 288  
 Leu Leu Tyr Gly Leu Phe Leu Lys Met Asn Arg Glu Gly Val Ile Ser  
 85 90 95

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ata gaa agt gat ata gaa aaa cct gaa tca agt cct atc ttt agt aaa 336  
 Ile Glu Ser Asp Ile Glu Lys Pro Glu Ser Ser Pro Ile Phe Ser Lys  
 100 105 110

25

tac cct aca att gta aaa gat act aaa gtt gtt gcc ttt att gca gat 384  
 Tyr Pro Thr Ile Val Lys Asp Thr Lys Val Val Ala Phe Ile Ala Asp  
 115 120 125

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aca tta cga gtt tat ctg aca aca ggt gca cca gaa gat ata gat aac 432  
 Thr Leu Arg Val Tyr Leu Thr Thr Gly Ala Pro Glu Asp Ile Asp Asn  
 130 135 140

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ctc atg gaa tct gac atg aaa att aca cac gaa gaa gaa tta tta cct 480  
 Leu Met Glu Ser Asp Met Lys Ile Thr His Glu Glu Glu Leu Leu Pro  
 145 150 155 160

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gca cat tcc atc agc cat atg gca gag tcg cta cca gga atg ggt att 528  
 Ala His Ser Ile Ser His Met Ala Glu Ser Leu Pro Gly Met Gly Ile  
 165 170 175

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gtt gct gca gta tta ggt gtt gtt att acc atg gga aaa att aat gag 576  
 Val Ala Ala Val Leu Gly Val Val Ile Thr Met Gly Lys Ile Asn Glu  
 180 185 190

cct cca gaa gtc ctt ggg cat tat att gga gca gct ttg gtt ggt aca 624  
 Pro Pro Glu Val Leu Gly His Tyr Ile Gly Ala Ala Leu Val Gly Thr  
 195 200 205

- 89 -

ttt ata ggt att ctt ttc tgt tat ggt ttt ttt gga cct atg ggt tca 672  
 Phe Ile Gly Ile Leu Phe Cys Tyr Gly Phe Phe Gly Pro Met Gly Ser  
 210 215 220

5 aag ctt gaa acc tct gca gaa gaa gca cat ttt tat tat aat tcc att 720  
 Lys Leu Glu Thr Ser Ala Glu Glu Ala His Phe Tyr Tyr Asn Ser Ile  
 225 230 235 240

10 aaa gaa gct gtt gca gct gct atc cga ggt tct aca cca atg ata gca 768  
 Lys Glu Ala Val Ala Ala Ala Ile Arg Gly Ser Thr Pro Met Ile Ala  
 245 250 255

15 gta gaa tat gga aga cgt gcc ata cct aat aca ttt cgt cca tca ttt 816  
 Val Glu Tyr Gly Arg Arg Ala Ile Pro Asn Thr Phe Arg Pro Ser Phe  
 260 265 270

tcg gaa atg gaa gaa cgt cta aaa aca gga taa 849  
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35 Glu Leu Val Ile Ile Ile Gly Ala Ala Leu Gly Ala Phe Phe Ala Ser  
 35 40 45

Gln Thr Lys Tyr Ser Phe Thr Leu Val Ile Lys Asn Leu Ser His Ile  
 50 55 60

40 Phe Gly Asp Pro Asn Ser Thr Lys Ile Lys Tyr Leu Glu Thr Leu Ala  
 65 70 75 80

Leu Leu Tyr Gly Leu Phe Leu Lys Met Asn Arg Glu Gly Val Ile Ser  
 85 90 95

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- 90 -

	Ile Glu Ser Asp Ile Glu Lys Pro Glu Ser Ser Pro Ile Phe Ser Lys	
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5	Tyr Pro Thr Ile Val Lys Asp Thr Lys Val Val Ala Phe Ile Ala Asp	
	115	120 125
	Thr Leu Arg Val Tyr Leu Thr Thr Gly Ala Pro Glu Asp Ile Asp Asn	
	130	135 140
10	Leu Met Glu Ser Asp Met Lys Ile Thr His Glu Glu Glu Leu Leu Pro	
	145	150 155 160
	Ala His Ser Ile Ser His Met Ala Glu Ser Leu Pro Gly Met Gly Ile	
	165	170 175
15	Val Ala Ala Val Leu Gly Val Val Ile Thr Met Gly Lys Ile Asn Glu	
	180	185 190
	Pro Pro Glu Val Leu Gly His Tyr Ile Gly Ala Ala Leu Val Gly Thr	
20	195	200 205
	Phe Ile Gly Ile Leu Phe Cys Tyr Gly Phe Phe Gly Pro Met Gly Ser	
	210	215 220
25	Lys Leu Glu Thr Ser Ala Glu Glu Ala His Phe Tyr Tyr Asn Ser Ile	
	225	230 235 240
	Lys Glu Ala Val Ala Ala Ala Ile Arg Gly Ser Thr Pro Met Ile Ala	
	245	250 255
30	Val Glu Tyr Gly Arg Arg Ala Ile Pro Asn Thr Phe Arg Pro Ser Phe	
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	atg gct ttc ttt cta ctg atg tgg att ctt gca atg aca ccc cct gag	96
	Met Ala Phe Phe Leu Leu Met Trp Ile Leu Ala Met Thr Pro Pro Glu	
	20 25 30	
10	gtt aaa gaa ggt ctt gct gca tat ttt tct tca tct gat gct aca ttt	144
	Val Lys Glu Gly Leu Ala Ala Tyr Phe Ser Ser Ser Asp Ala Thr Phe	
	35 40 45	
15	aaa aca cct gat agt tcg cca atc tct aac aat cct ctt atc aac caa	192
	Lys Thr Pro Asp Ser Ser Pro Ile Ser Asn Asn Pro Leu Ile Asn Gln	
	50 55 60	
	ata gat aaa ctt gat act cga caa tta aaa att aat gaa aca gaa caa	240
20	Ile Asp Lys Leu Asp Thr Arg Gln Leu Lys Ile Asn Glu Thr Glu Gln	
	65 70 75 80	
	tct cat tat gct ctt gct aat aaa tta aaa aaa atg tta atg gct gat	288
	Ser His Tyr Ala Leu Ala Asn Lys Leu Lys Lys Met Leu Met Ala Asp	
25	85 90 95	
	gct atc cca cag tca gca aca gga ata agt gct gac gat gtt ggt gta	336
	Ala Ile Pro Gln Ser Ala Thr Gly Ile Ser Ala Asp Asp Val Gly Val	
	100 105 110	
30	tta tta cgt gta aat tct aat tcc acg ttt ttt cct ggt aca gca act	384
	Leu Leu Arg Val Asn Ser Asn Ser Thr Phe Phe Pro Gly Thr Ala Thr	
	115 120 125	
35	ctt aca ccc gaa ggg aaa aaa gtt atg gga act gtt tta gcc gtt ctc	432
	Leu Thr Pro Glu Gly Lys Lys Val Met Gly Thr Val Leu Ala Val Leu	
	130 135 140	
	cgt gaa tat aat ctt tac ctt gtg ata cgt ggc cat gct gat att ggt	480
40	Arg Glu Tyr Asn Leu Tyr Leu Val Ile Arg Gly His Ala Asp Ile Gly	
	145 150 155 160	
	gaa ata aca aaa ggc agc cct ttt gct tct aac tgg gaa ctt tca gga	528
	Glu Ile Thr Lys Gly Ser Pro Phe Ala Ser Asn Trp Glu Leu Ser Gly	
45	165 170 175	

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gct cgt gca gct gca gct gca cag tat ctt gta gag cac ggg ata aag 576  
 Ala Arg Ala Ala Ala Ala Ala Gln Tyr Leu Val Glu His Gly Ile Lys  
 180 185 190

5

gct tca cga att cgc tct gta gga tat gca gat aca aga cct cta gaa 624  
 Ala Ser Arg Ile Arg Ser Val Gly Tyr Ala Asp Thr Arg Pro Leu Glu  
 195 200 205

10

cct agt tct cct gaa gga agt aca aaa aat cgt cgt ata gaa ttc tat 672  
 Pro Ser Ser Pro Glu Gly Ser Thr Lys Asn Arg Arg Ile Glu Phe Tyr  
 210 215 220

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ttt cat cgg cca gaa gtt atg tct tat ggc gtt gta tat taa tag 717  
 Phe His Arg Pro Glu Val Met Ser Tyr Gly Val Val Tyr  
 225 230 235

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 <212> PRT  
 <213> *Lawsonia intracellularis*

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Met Ala Phe Phe Leu Leu Met Trp Ile Leu Ala Met Thr Pro Pro Glu  
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Val Lys Glu Gly Leu Ala Ala Tyr Phe Ser Ser Ser Asp Ala Thr Phe  
 35 40 45

35

Lys Thr Pro Asp Ser Ser Pro Ile Ser Asn Asn Pro Leu Ile Asn Gln  
 50 55 60

Ile Asp Lys Leu Asp Thr Arg Gln Leu Lys Ile Asn Glu Thr Glu Gln  
 65 70 75 80

40

Ser His Tyr Ala Leu Ala Asn Lys Leu Lys Lys Met Leu Met Ala Asp  
 85 90 95

45

Ala Ile Pro Gln Ser Ala Thr Gly Ile Ser Ala Asp Asp Val Gly Val  
 100 105 110

- 93 -

Leu Leu Arg Val Asn Ser Asn Ser Thr Phe Phe Pro Gly Thr Ala Thr  
 115 120 125

5 Leu Thr Pro Glu Gly Lys Lys Val Met Gly Thr Val Leu Ala Val Leu  
 130 135 140

Arg Glu Tyr Asn Leu Tyr Leu Val Ile Arg Gly His Ala Asp Ile Gly  
 145 150 155 160

10 Glu Ile Thr Lys Gly Ser Pro Phe Ala Ser Asn Trp Glu Leu Ser Gly  
 165 170 175

Ala Arg Ala Ala Ala Ala Ala Gln Tyr Leu Val Glu His Gly Ile Lys  
 180 185 190

15 Ala Ser Arg Ile Arg Ser Val Gly Tyr Ala Asp Thr Arg Pro Leu Glu  
 195 200 205

20 Pro Ser Ser Pro Glu Gly Ser Thr Lys Asn Arg Arg Ile Glu Phe Tyr  
 210 215 220

Phe His Arg Pro Glu Val Met Ser Tyr Gly Val Val Tyr  
 225 230 235

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30 <220>  
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 Met Ile Ile Leu Leu Gly Thr Val Phe Leu Ile Val Leu Ile Ser Ala  
 1 5 10 15

40 tta tgc tca atg atg gaa gct gct ata tac tct atc cct att act tat 96  
 Leu Cys Ser Met Met Glu Ala Ala Ile Tyr Ser Ile Pro Ile Thr Tyr  
 20 25 30

45 att gaa cac ctt cgt gaa cag gga agc aaa aaa gga gaa aaa ctt tat 144  
 Ile Glu His Leu Arg Glu Gln Gly Ser Lys Lys Gly Glu Lys Leu Tyr

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	35	40	45	
	tat tta cat agt aat att gat cag cct att aca gcc gta tta ata ttg			192
	Tyr Leu His Ser Asn Ile Asp Gln Pro Ile Thr Ala Val Leu Ile Leu			
5	50	55	60	
	aat act ata gca aat act gct gga gct gcc ctt gct gga gca att gct			240
	Asn Thr Ile Ala Asn Thr Ala Gly Ala Ala Leu Ala Gly Ala Ile Ala			
10	65	70	75	80
	aca aca aca ctt cat gaa tct act aag cct ttc ttt gca gca atc ctc			288
	Thr Thr Thr Leu His Glu Ser Thr Lys Pro Phe Phe Ala Ala Ile Leu			
	85	90	95	
15	acc ttg ctt att tta gct ttt ggg gaa att ata cct aaa aca cta ggt			336
	Thr Leu Leu Ile Leu Ala Phe Gly Glu Ile Ile Pro Lys Thr Leu Gly			
	100	105	110	
	gtt gct tac tct aaa cgt att gct ata att ctc ctt aat cct ctc tct			384
20	Val Ala Tyr Ser Lys Arg Ile Ala Ile Ile Leu Leu Asn Pro Leu Ser			
	115	120	125	
	att ctt ata gtt act tta aaa ccc ctt att atg ctt tca agc tac tta			432
	Ile Leu Ile Val Thr Leu Lys Pro Leu Ile Met Leu Ser Ser Tyr Leu			
25	130	135	140	
	aca cga ctt gtt tca cct cga aaa cgt cct aca gtt aca gaa gat gac			480
	Thr Arg Leu Val Ser Pro Arg Lys Arg Pro Thr Val Thr Glu Asp Asp			
30	145	150	155	160
	atc cgt gca ctt aca agt ctt tcc aga gag tct ggt cgt att aag cca			528
	Ile Arg Ala Leu Thr Ser Leu Ser Arg Glu Ser Gly Arg Ile Lys Pro			
	165	170	175	
35	tat gaa gaa cat gtc ata aaa aat atc ctt agt ctt gat tta aaa tat			576
	Tyr Glu Glu His Val Ile Lys Asn Ile Leu Ser Leu Asp Leu Lys Tyr			
	180	185	190	
	gct cat gaa att atg act ccc aga act atg gtc ttt tca ctt cat gaa			624
40	Ala His Glu Ile Met Thr Pro Arg Thr Met Val Phe Ser Leu His Glu			
	195	200	205	
	aac ctt act gtc tct gaa gct tat agc aac ccc aaa ata tgg aac tat			672
	Asn Leu Thr Val Ser Glu Ala Tyr Ser Asn Pro Lys Ile Trp Asn Tyr			
45	210	215	220	

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agt cgc atc cct act tat gga gaa aat aac gaa gac att act ggc att 720  
 Ser Arg Ile Pro Thr Tyr Gly Glu Asn Asn Glu Asp Ile Thr Gly Ile  
 225 230 235 240

5

atc caa cga tat gaa att gga cga tat atg acc aat gga gaa aca gaa 768  
 Ile Gln Arg Tyr Glu Ile Gly Arg Tyr Met Thr Asn Gly Glu Thr Glu  
 245 250 255

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aaa aaa ctt tta gaa att atg caa cca gca aaa ttt gtc ctt gaa agt 816  
 Lys Lys Leu Leu Glu Ile Met Gln Pro Ala Lys Phe Val Leu Glu Ser  
 260 265 270

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caa act gta gat cat tta ctt ctt gca ttt tta gaa gaa aga caa cat 864  
 Gln Thr Val Asp His Leu Leu Leu Ala Phe Leu Glu Glu Arg Gln His  
 275 280 285

20

ctt ttt att gta ctt gat gag tat ggg gga tta tct ggt gtt gtt tcc 912  
 Leu Phe Ile Val Leu Asp Glu Tyr Gly Gly Leu Ser Gly Val Val Ser  
 290 295 300

25

tta gaa gat gta tta gaa act atg ctt gga aga gaa att gtt gat gaa 960  
 Leu Glu Asp Val Leu Glu Thr Met Leu Gly Arg Glu Ile Val Asp Glu  
 305 310 315 320

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agt gat aca aca cct gat ctt aga gca ctt gca aaa aaa aga cat agt 1008  
 Ser Asp Thr Thr Pro Asp Leu Arg Ala Leu Ala Lys Lys Arg His Ser  
 325 330 335

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gca tta atc caa aat aat aaa aat act ctt tta aaa taa 1047  
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 1 5 10 15

45

Leu Cys Ser Met Met Glu Ala Ala Ile Tyr Ser Ile Pro Ile Thr Tyr  
 20 25 30

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Ile Glu His Leu Arg Glu Gln Gly Ser Lys Lys Gly Glu Lys Leu Tyr  
 35 40 45

5 Tyr Leu His Ser Asn Ile Asp Gln Pro Ile Thr Ala Val Leu Ile Leu  
 50 55 60

Asn Thr Ile Ala Asn Thr Ala Gly Ala Ala Leu Ala Gly Ala Ile Ala  
 65 70 75 80

10 Thr Thr Thr Leu His Glu Ser Thr Lys Pro Phe Phe Ala Ala Ile Leu  
 85 90 95

Thr Leu Leu Ile Leu Ala Phe Gly Glu Ile Ile Pro Lys Thr Leu Gly  
 15 100 105 110

Val Ala Tyr Ser Lys Arg Ile Ala Ile Ile Leu Leu Asn Pro Leu Ser  
 115 120 125

20 Ile Leu Ile Val Thr Leu Lys Pro Leu Ile Met Leu Ser Ser Tyr Leu  
 130 135 140

Thr Arg Leu Val Ser Pro Arg Lys Arg Pro Thr Val Thr Glu Asp Asp  
 145 150 155 160

25 Ile Arg Ala Leu Thr Ser Leu Ser Arg Glu Ser Gly Arg Ile Lys Pro  
 165 170 175

Tyr Glu Glu His Val Ile Lys Asn Ile Leu Ser Leu Asp Leu Lys Tyr  
 30 180 185 190

Ala His Glu Ile Met Thr Pro Arg Thr Met Val Phe Ser Leu His Glu  
 195 200 205

35 Asn Leu Thr Val Ser Glu Ala Tyr Ser Asn Pro Lys Ile Trp Asn Tyr  
 210 215 220

Ser Arg Ile Pro Thr Tyr Gly Glu Asn Asn Glu Asp Ile Thr Gly Ile  
 225 230 235 240

40 Ile Gln Arg Tyr Glu Ile Gly Arg Tyr Met Thr Asn Gly Glu Thr Glu  
 245 250 255

Lys Lys Leu Leu Glu Ile Met Gln Pro Ala Lys Phe Val Leu Glu Ser  
 45 260 265 270

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Gln Thr Val Asp His Leu Leu Leu Ala Phe Leu Glu Glu Arg Gln His  
 275 280 285

5 Leu Phe Ile Val Leu Asp Glu Tyr Gly Gly Leu Ser Gly Val Val Ser  
 290 295 300

Leu Glu Asp Val Leu Glu Thr Met Leu Gly Arg Glu Ile Val Asp Glu  
 305 310 315 320

10 Ser Asp Thr Thr Pro Asp Leu Arg Ala Leu Ala Lys Lys Arg His Ser  
 325 330 335

Ala Leu Ile Gln Asn Asn Lys Asn Thr Leu Leu Lys  
 15 340 345

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 20 <212> DNA  
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 25 <222> (1)..(1809)

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 Met Gln Lys Val Cys Tyr Phe Phe Leu Ile Thr Phe Phe Tyr Phe Phe  
 30 1 5 10 15

ata aca gaa aat tat ctc ttt gct aca tca att acc act tcc aca att 96  
 Ile Thr Glu Asn Tyr Leu Phe Ala Thr Ser Ile Thr Thr Ser Thr Ile  
 20 25 30

35 aac caa caa cat ata gca tat aca gtt act ttt acc tct cca gaa aat 144  
 Asn Gln Gln His Ile Ala Tyr Thr Val Thr Phe Thr Ser Pro Glu Asn  
 35 40 45

40 cct aat ctt gca aca gag atg gaa aca cat agt gaa tta gta aag ctt 192  
 Pro Asn Leu Ala Thr Glu Met Glu Thr His Ser Glu Leu Val Lys Leu  
 50 55 60

gca aat caa tct tta gat agt aaa ata ggt tta aat tta cgt gtt aaa 240  
 45 Ala Asn Gln Ser Leu Asp Ser Lys Ile Gly Leu Asn Leu Arg Val Lys

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	65	70	75	80	
	gaa gat ata agt aca gca caa aaa att ctt gac tcg aat ggt tat tat				288
5	Glu Asp Ile Ser Thr Ala Gln Lys Ile Leu Asp Ser Asn Gly Tyr Tyr				
	85		90	95	
	agt gga agt gtc gag gga aag att gac tgg cag acg aac cct att agt				336
	Ser Gly Ser Val Glu Gly Lys Ile Asp Trp Gln Thr Asn Pro Ile Ser				
10	100		105	110	
	atc caa atc caa ttt aaa cca aat gta caa tat aaa ata aat aca ata				384
	Ile Gln Ile Gln Phe Lys Pro Asn Val Gln Tyr Lys Ile Asn Thr Ile				
	115		120	125	
15	cat atc caa tac ctt gat agt gaa ctt gca tat ctc cct ctt tcc tta				432
	His Ile Gln Tyr Leu Asp Ser Glu Leu Ala Tyr Leu Pro Leu Ser Leu				
	130		135	140	
	gaa gaa ttc aat ctc tct aaa ggt aat cct gct ctt gct gtt aat atc				480
20	Glu Glu Phe Asn Leu Ser Lys Gly Asn Pro Ala Leu Ala Val Asn Ile				
	145		150	155	160
	cta tcc tct gta agt agc ctc atg caa tat ata cat aat aat gga tat				528
25	Leu Ser Ser Val Ser Ser Leu Met Gln Tyr Ile His Asn Asn Gly Tyr				
	165		170	175	
	cca tta gcc aaa ata aaa aaa act caa tac ata att aat cgg atg gat				576
	Pro Leu Ala Lys Ile Lys Lys Thr Gln Tyr Ile Ile Asn Arg Met Asp				
30	180		185	190	
	tat aca ttt gat att gat tta gta ata aga caa gga ccg tta ctc cat				624
	Tyr Thr Phe Asp Ile Asp Leu Val Ile Arg Gln Gly Pro Leu Leu His				
	195		200	205	
35	atg ggt aaa gta caa cct caa cat aat ctc aat att tca aca ata ttc				672
	Met Gly Lys Val Gln Pro Gln His Asn Leu Asn Ile Ser Thr Ile Phe				
	210		215	220	
	cta aat aaa att gct aca tgg aag gaa gga agg gta tgg aac aat gca				720
40	Leu Asn Lys Ile Ala Thr Trp Lys Glu Gly Arg Val Trp Asn Asn Ala				
	225		230	235	240
	ctc ctt gat tct tat cga aca cgg ctt caa caa aca ggc ctt ttc agt				768
45	Leu Leu Asp Ser Tyr Arg Thr Arg Leu Gln Gln Thr Gly Leu Phe Ser				
	245		250	255	

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	tct ata act ctc aat cca agg aat caa aaa gaa caa aat ggt aac acc	816
	Ser Ile Thr Leu Asn Pro Arg Asn Gln Lys Glu Gln Asn Gly Asn Thr	
	260 265 270	
5		
	tct ata gaa ctt gtt gca aca gaa gcc cct cca agg act att agt ggt	864
	Ser Ile Glu Leu Val Ala Thr Glu Ala Pro Pro Arg Thr Ile Ser Gly	
	275 280 285	
10		
	ggc tta caa tac tct tct gat caa ggt att ggt gca cgt ggg act tgg	912
	Gly Leu Gln Tyr Ser Ser Asp Gln Gly Ile Gly Ala Arg Gly Thr Trp	
	290 295 300	
	gaa cat cga aat gtt ttt ggt aat gga gaa ctt ttt cgt ata aca gca	960
15	Glu His Arg Asn Val Phe Gly Asn Gly Glu Leu Phe Arg Ile Thr Ala	
	305 310 315 320	
	cca ata agt cga gat gat caa aaa att atg gca aac ttc caa aaa cca	1008
	Pro Ile Ser Arg Asp Asp Gln Lys Ile Met Ala Asn Phe Gln Lys Pro	
20	325 330 335	
	gcc ttt ggc cgt cca aat caa tca tta att agt gaa gca caa ctt aaa	1056
	Ala Phe Gly Arg Pro Asn Gln Ser Leu Ile Ser Glu Ala Gln Leu Lys	
	340 345 350	
25		
	aaa gaa aat aca aaa agt tac aaa caa caa ctt gca tct att gct tta	1104
	Lys Glu Asn Thr Lys Ser Tyr Lys Gln Gln Leu Ala Ser Ile Ala Leu	
	355 360 365	
30		
	gga att gaa cga caa ttt aat aga cgt tgg ttt ggt agt agc agt ctt	1152
	Gly Ile Glu Arg Gln Phe Asn Arg Arg Trp Phe Gly Ser Ser Ser Leu	
	370 375 380	
	tca gtt gat aca gga ttt atg gat gat cga gat tct ata aaa aaa ata	1200
35	Ser Val Asp Thr Gly Phe Met Asp Asp Arg Asp Ser Ile Lys Lys Ile	
	385 390 395 400	
	ttt act ctt ttt ggc atc ccc tta tca ata aca agg gat agt tct aaa	1248
	Phe Thr Leu Phe Gly Ile Pro Leu Ser Ile Thr Arg Asp Ser Ser Lys	
40	405 410 415	
	gat cct ctt aat cct atc caa gga aca aaa gct acc tta aat gtt act	1296
	Asp Pro Leu Asn Pro Ile Gln Gly Thr Lys Ala Thr Leu Asn Val Thr	
	420 425 430	
45		

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	cct tat att ggt aaa tat aaa aaa aag att ttg act tta cgt agt cgg	1344
	Pro Tyr Ile Gly Lys Tyr Lys Lys Lys Ile Leu Thr Leu Arg Ser Arg	
	435 440 445	
5	ttt gat ttt agc ttt tac ata gac gtt ctt aaa aca ggg aaa ctt atc	1392
	Phe Asp Phe Ser Phe Tyr Ile Asp Val Leu Lys Thr Gly Lys Leu Ile	
	450 455 460	
10	ttg gct aac aaa ata gca ata ggt tcc ctc cta ggg aaa gat ata gaa	1440
	Leu Ala Asn Lys Ile Ala Ile Gly Ser Leu Leu Gly Lys Asp Ile Glu	
	465 470 475 480	
15	aac tat cct gca ata cta agg ttt tat gct ggg ggt ggt ggt agt gta	1488
	Asn Tyr Pro Ala Ile Leu Arg Phe Tyr Ala Gly Gly Gly Ser Val	
	485 490 495	
20	aga ggg tat gac tat caa tca ttg gga cca aaa aat aaa tat ggg gat	1536
	Arg Gly Tyr Asp Tyr Gln Ser Leu Gly Pro Lys Asn Lys Tyr Gly Asp	
	500 505 510	
25	gct att gga gga ctt tct ttt tca act att agt ttt gaa tta cga tta	1584
	Ala Ile Gly Gly Leu Ser Phe Ser Thr Ile Ser Phe Glu Leu Arg Leu	
	515 520 525	
30	aaa ata aca gaa tcc att ggc att gtg cca att tat tgg atg ggg gaa	1632
	Lys Ile Thr Glu Ser Ile Gly Ile Val Pro Ile Tyr Trp Met Gly Glu	
	530 535 540	
35	tat tta cga aaa aaa aat ttc ctg act tta aaa aaa tca ata tat tgg	1680
	Tyr Leu Arg Lys Lys Asn Phe Leu Thr Leu Lys Lys Ser Ile Tyr Trp	
	545 550 555 560	
40	ggg gta ggc ctg ggg cta cga tat tat aca agt ttt gcc ccc ata cgt	1728
	Gly Val Gly Leu Gly Leu Arg Tyr Tyr Thr Ser Phe Ala Pro Ile Arg	
	565 570 575	
45	tta gat ata gca act cca ctt caa gat aga agc cat aat aaa cac ttt	1776
	Leu Asp Ile Ala Thr Pro Leu Gln Asp Arg Ser His Asn Lys His Phe	
	580 585 590	
50	caa ctt tat att agt att ggg caa gca ttc taa tga	1812
	Gln Leu Tyr Ile Ser Ile Gly Gln Ala Phe	
	595 600	

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<210> 16  
 <211> 602  
 <212> PRT  
 <213> Lawsonia intracellularis

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 Met Gln Lys Val Cys Tyr Phe Phe Leu Ile Thr Phe Phe Tyr Phe Phe  
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 20 25 30

Asn Gln Gln His Ile Ala Tyr Thr Val Thr Phe Thr Ser Pro Glu Asn  
 35 40 45

15

Pro Asn Leu Ala Thr Glu Met Glu Thr His Ser Glu Leu Val Lys Leu  
 50 55 60

20 Ala Asn Gln Ser Leu Asp Ser Lys Ile Gly Leu Asn Leu Arg Val Lys  
 65 70 75 80

Glu Asp Ile Ser Thr Ala Gln Lys Ile Leu Asp Ser Asn Gly Tyr Tyr  
 85 90 95

25 Ser Gly Ser Val Glu Gly Lys Ile Asp Trp Gln Thr Asn Pro Ile Ser  
 100 105 110

Ile Gln Ile Gln Phe Lys Pro Asn Val Gln Tyr Lys Ile Asn Thr Ile  
 115 120 125

30

His Ile Gln Tyr Leu Asp Ser Glu Leu Ala Tyr Leu Pro Leu Ser Leu  
 130 135 140

35 Glu Glu Phe Asn Leu Ser Lys Gly Asn Pro Ala Leu Ala Val Asn Ile  
 145 150 155 160

Leu Ser Ser Val Ser Ser Leu Met Gln Tyr Ile His Asn Asn Gly Tyr  
 165 170 175

40 Pro Leu Ala Lys Ile Lys Lys Thr Gln Tyr Ile Ile Asn Arg Met Asp  
 180 185 190

Tyr Thr Phe Asp Ile Asp Leu Val Ile Arg Gln Gly Pro Leu Leu His  
 195 200 205

45

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Met Gly Lys Val Gln Pro Gln His Asn Leu Asn Ile Ser Thr Ile Phe  
 210 215 220

5 Leu Asn Lys Ile Ala Thr Trp Lys Glu Gly Arg Val Trp Asn Asn Ala  
 225 230 235 240

Leu Leu Asp Ser Tyr Arg Thr Arg Leu Gln Gln Thr Gly Leu Phe Ser  
 245 250 255

10 Ser Ile Thr Leu Asn Pro Arg Asn Gln Lys Glu Gln Asn Gly Asn Thr  
 260 265 270

Ser Ile Glu Leu Val Ala Thr Glu Ala Pro Pro Arg Thr Ile Ser Gly  
 275 280 285

15 Gly Leu Gln Tyr Ser Ser Asp Gln Gly Ile Gly Ala Arg Gly Thr Trp  
 290 295 300

Glu His Arg Asn Val Phe Gly Asn Gly Glu Leu Phe Arg Ile Thr Ala  
 20 305 310 315 320

Pro Ile Ser Arg Asp Asp Gln Lys Ile Met Ala Asn Phe Gln Lys Pro  
 325 330 335

25 Ala Phe Gly Arg Pro Asn Gln Ser Leu Ile Ser Glu Ala Gln Leu Lys  
 340 345 350

Lys Glu Asn Thr Lys Ser Tyr Lys Gln Gln Leu Ala Ser Ile Ala Leu  
 355 360 365

30 Gly Ile Glu Arg Gln Phe Asn Arg Arg Trp Phe Gly Ser Ser Ser Leu  
 370 375 380

Ser Val Asp Thr Gly Phe Met Asp Asp Arg Asp Ser Ile Lys Lys Ile  
 35 385 390 395 400

Phe Thr Leu Phe Gly Ile Pro Leu Ser Ile Thr Arg Asp Ser Ser Lys  
 405 410 415

40 Asp Pro Leu Asn Pro Ile Gln Gly Thr Lys Ala Thr Leu Asn Val Thr  
 420 425 430

Pro Tyr Ile Gly Lys Tyr Lys Lys Lys Ile Leu Thr Leu Arg Ser Arg  
 435 440 445

45

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Phe Asp Phe Ser Phe Tyr Ile Asp Val Leu Lys Thr Gly Lys Leu Ile  
 450 455 460

5 Leu Ala Asn Lys Ile Ala Ile Gly Ser Leu Leu Gly Lys Asp Ile Glu  
 465 470 475 480

Asn Tyr Pro Ala Ile Leu Arg Phe Tyr Ala Gly Gly Gly Gly Ser Val  
 485 490 495

10 Arg Gly Tyr Asp Tyr Gln Ser Leu Gly Pro Lys Asn Lys Tyr Gly Asp  
 500 505 510

Ala Ile Gly Gly Leu Ser Phe Ser Thr Ile Ser Phe Glu Leu Arg Leu  
 515 520 525

15 Lys Ile Thr Glu Ser Ile Gly Ile Val Pro Ile Tyr Trp Met Gly Glu  
 530 535 540

Tyr Leu Arg Lys Lys Asn Phe Leu Thr Leu Lys Lys Ser Ile Tyr Trp  
 20 545 550 555 560

Gly Val Gly Leu Gly Leu Arg Tyr Tyr Thr Ser Phe Ala Pro Ile Arg  
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25 Leu Asp Ile Ala Thr Pro Leu Gln Asp Arg Ser His Asn Lys His Phe  
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Gln Leu Tyr Ile Ser Ile Gly Gln Ala Phe  
 595 600

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 <212> DNA  
 <213> Lawsonia intracellularis

35 <220>  
 <221> CDS  
 <222> (1)..(4146)

40 <400> 17  
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 Met Asn Asn Thr Lys Ile Leu Ser Lys Leu Leu Tyr Thr Leu Leu Gly  
 1 5 10 15

45 gca ttt acg tta ttt tta gga ctt att att aca ggc att ctt ttt ata 96

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	Ala Phe Thr Leu Phe Leu Gly Leu Ile Ile Thr Gly Ile Leu Phe Ile	
	20 25 30	
5	cgg acc tct aca ggc att gct tgg att aaa aat aca gtt tct tct tta Arg Thr Ser Thr Gly Ile Ala Trp Ile Lys Asn Thr Val Ser Ser Leu	144
	35 40 45	
10	ctt caa caa caa gga att ata cta caa gta tct tca att att gga cca Leu Gln Gln Gln Gly Ile Ile Leu Gln Val Ser Ser Ile Ile Gly Pro	192
	50 55 60	
15	ttc cca gaa caa att act att aat gaa ctt agc ctt agt gat gtg aat Phe Pro Glu Gln Ile Thr Ile Asn Glu Leu Ser Leu Ser Asp Val Asn	240
	65 70 75 80	
	gga act tac ctt aca ata tct aac tta gaa atc caa tca aac tta tgg Gly Thr Tyr Leu Thr Ile Ser Asn Leu Glu Ile Gln Ser Asn Leu Trp	288
	85 90 95	
20	gct tta ttc aaa ggt caa ctt gaa att ctg tct ttt gaa ctt aat gat Ala Leu Phe Lys Gly Gln Leu Glu Ile Leu Ser Phe Glu Leu Asn Asp	336
	100 105 110	
25	ctt gta tta tat cgc tta ccc tca aat aat aat cta aaa aaa tca tct Leu Val Leu Tyr Arg Leu Pro Ser Asn Asn Asn Leu Lys Lys Ser Ser	384
	115 120 125	
30	aca agt ttt gtg tta cct cac ata tca ttt gat tta act cca tgg tgg Thr Ser Phe Val Leu Pro His Ile Ser Phe Asp Leu Thr Pro Trp Trp	432
	130 135 140	
35	act gaa cat att cgt att caa aac atc cat att aac aat aca caa ctt Thr Glu His Ile Arg Ile Gln Asn Ile His Ile Asn Asn Thr Gln Leu	480
	145 150 155 160	
	tcc tct gat att ata ggt att cca ttg gta tta tcc ctt gag ggt gat Ser Ser Asp Ile Ile Gly Ile Pro Leu Val Leu Ser Leu Glu Gly Asp	528
	165 170 175	
40	ggt aca tta aca aat tgg aat gga aca ttt caa cta tcc tct tct aac Gly Thr Leu Thr Asn Trp Asn Gly Thr Phe Gln Leu Ser Ser Ser Asn	576
	180 185 190	
45	aaa aca aaa att ata gga acg ctt cgt tac caa ggg aat aag aca caa Lys Thr Lys Ile Ile Gly Thr Leu Arg Tyr Gln Gly Asn Lys Thr Gln	624



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	195	200	205	
	ttt ttt gaa tat gtt cat cct aca cgg ata gta aca cta gag ata gac	672		
	Phe Phe Glu Tyr Val His Pro Thr Arg Ile Val Thr Leu Glu Ile Asp			
5	210	215	220	
	agc gta gct gat aaa aag tca tat aat aat agt atc ctt gaa caa cct	720		
	Ser Val Ala Asp Lys Lys Ser Tyr Asn Asn Ser Ile Leu Glu Gln Pro			
	225	230	235	240
10				
	cta cat tta cac ctt tct att tat cct gaa cat aat aga att atc tta	768		
	Leu His Leu His Leu Ser Ile Tyr Pro Glu His Asn Arg Ile Ile Leu			
	245	250	255	
15				
	cac tca tta cta gct gaa tat ggt agc tgg tta ctt aca tca gaa agt	816		
	His Ser Leu Leu Ala Glu Tyr Gly Ser Trp Leu Leu Thr Ser Glu Ser			
	260	265	270	
20				
	att gaa gta tct aat gag caa tta aaa gga aat att tta tta aaa tat	864		
	Ile Glu Val Ser Asn Glu Gln Leu Lys Gly Asn Ile Leu Leu Lys Tyr			
	275	280	285	
25				
	aat gga gaa gct act cat caa ctt cct ata aaa aaa ctt aac tca tca	912		
	Asn Gly Glu Ala Thr His Gln Leu Pro Ile Lys Lys Leu Asn Ser Ser			
	290	295	300	
30				
	att acc ctc agt ggc tca cta aat aaa cct aat ttt agt ata caa atg	960		
	Ile Thr Leu Ser Gly Ser Leu Asn Lys Pro Asn Phe Ser Ile Gln Met			
	305	310	315	320
	aca tta cct gaa att aac att aca aaa aac ata ata gat ctt caa aca	1008		
	Thr Leu Pro Glu Ile Asn Ile Thr Lys Asn Ile Ile Asp Leu Gln Thr			
	325	330	335	
35				
	gaa ctt gtt att aat cta gga ctt ttc tct act cac tct gat att ctt	1056		
	Glu Leu Val Ile Asn Leu Gly Leu Phe Ser Thr His Ser Asp Ile Leu			
	340	345	350	
40				
	aca tct ggg aca att aca gta cag gga gaa act ata ccc aat agt att	1104		
	Thr Ser Gly Thr Ile Thr Val Gln Gly Glu Thr Ile Pro Asn Ser Ile			
	355	360	365	
45				
	ctt tcc agt gca gtt gat ata ata gcc tct aca aca aca cat aca att	1152		
	Leu Ser Ser Ala Val Asp Ile Ile Ala Ser Thr Thr Thr His Thr Ile			
	370	375	380	

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acc tta gag cat gca acc tta aca tct cca gaa atg cat ttt tcc cta 1200  
 Thr Leu Glu His Ala Thr Leu Thr Ser Pro Glu Met His Phe Ser Leu  
 385 390 395 400  
 5

tct gga gaa ttt aat agt ctt cta gga aat atc gat gca aac cta aaa 1248  
 Ser Gly Glu Phe Asn Ser Leu Leu Gly Asn Ile Asp Ala Asn Leu Lys  
 405 410 415

10 ggt aat act cca act ctt agt ata ttt tct tct ctt ctt gga cta cct 1296  
 Gly Asn Thr Pro Thr Leu Ser Ile Phe Ser Ser Leu Leu Gly Leu Pro  
 420 425 430

15 gat ctt act ggg caa agt aac att act ata gga tta cac cgt caa ggg 1344  
 Asp Leu Thr Gly Gln Ser Asn Ile Thr Ile Gly Leu His Arg Gln Gly  
 435 440 445

20 tct tcc tct tca ata gaa gga aca gca act gtc tca ctt aat aat atg 1392  
 Ser Ser Ser Ser Ile Glu Gly Thr Ala Thr Val Ser Leu Asn Asn Met  
 450 455 460

25 aac tgg gga gta caa gca tta cag ggg aca tta ggt gat aat gca act 1440  
 Asn Trp Gly Val Gln Ala Leu Gln Gly Thr Leu Gly Asp Asn Ala Thr  
 465 470 475 480

cta agt gga ata tat aat tta act ccc ata gac tgg tct att tct tta 1488  
 Leu Ser Gly Ile Tyr Asn Leu Thr Pro Ile Asp Trp Ser Ile Ser Leu  
 485 490 495

30 aac aaa ttg aaa tta aca gca aag aat gtt tat gct gaa ggc ctt att 1536  
 Asn Lys Leu Lys Leu Thr Ala Lys Asn Val Tyr Ala Glu Gly Leu Ile  
 500 505 510

35 aat ttt caa aaa aaa tac ata gat agc tct ata aat ctt ata att cct 1584  
 Asn Phe Gln Lys Lys Tyr Ile Asp Ser Ser Ile Asn Leu Ile Ile Pro  
 515 520 525

40 aac ctt cag cta ata gct cct cct ata tct gga gag tta caa tcc tta 1632  
 Asn Leu Gln Leu Ile Ala Pro Pro Ile Ser Gly Glu Leu Gln Ser Leu  
 530 535 540

45 att aca gtg tct gga aaa ctt gac gca cct tct ata gaa agc aaa att 1680  
 Ile Thr Val Ser Gly Lys Leu Asp Ala Pro Ser Ile Glu Ser Lys Ile  
 545 550 555 560



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	ttt tca tca caa ctc acc tgg aat gcg ctc caa ctt aat aat cct caa	1728
	Phe Ser Ser Gln Leu Thr Trp Asn Ala Leu Gln Leu Asn Asn Pro Gln	
	565 570 575	
5	ctc ata ata act act act caa tct tct tcc tct gcg att aaa ggt aat	1776
	Leu Ile Ile Thr Thr Thr Gln Ser Ser Ser Ser Ala Ile Lys Gly Asn	
	580 585 590	
10	ata aca ctc tcg gct gag cca gct tca tct gag gca tta acc ttt tca	1824
	Ile Thr Leu Ser Ala Glu Pro Ala Ser Ser Glu Ala Leu Thr Phe Ser	
	595 600 605	
15	agt aat tgg gga atc cta cct acg gaa ata cta gta gaa aaa att ata	1872
	Ser Asn Trp Gly Ile Leu Pro Thr Glu Ile Leu Val Glu Lys Ile Ile	
	610 615 620	
20	gga aat ata tta gga gta aat ctt gat ggt aat att aaa ata aca aaa	1920
	Gly Asn Ile Leu Gly Val Asn Leu Asp Gly Asn Ile Lys Ile Thr Lys	
	625 630 635 640	
25	aaa gat tac ctt ata aat ggt gat att att gca gaa gtt cag tct tgg	1968
	Lys Asp Tyr Leu Ile Asn Gly Asp Ile Ile Ala Glu Val Gln Ser Trp	
	645 650 655	
30	ata aaa ata cag ttt gat cca aag aat caa caa tgt att tct act caa	2064
	Ile Lys Ile Gln Phe Asp Pro Lys Asn Gln Gln Cys Ile Ser Thr Gln	
	675 680 685	
35	tgg caa tta aaa aat ttc ata tta ggt aat aat ttt aat gta act act	2112
	Trp Gln Leu Lys Asn Phe Ile Leu Gly Asn Asn Phe Asn Val Thr Thr	
	690 695 700	
40	ata aaa gga aga gca gat aca ata caa ctt cat aag aat cct aca att	2160
	Ile Lys Gly Arg Ala Asp Thr Ile Gln Leu His Lys Asn Pro Thr Ile	
	705 710 715 720	
45	gct ctc tct tca aaa att ggt gct ggt aca tat gaa gac ttt caa tgg	2208
	Ala Leu Ser Ser Lys Ile Gly Ala Gly Thr Tyr Glu Asp Phe Gln Trp	
	725 730 735	
45	aca caa ggg acg tta gac ata aaa ggc aca tta aaa aat ttt aat agt	2256

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	Thr	Gln	Gly	Thr	Leu	Asp	Ile	Lys	Gly	Thr	Leu	Lys	Asn	Phe	Asn	Ser	
				740					745					750			
	aaa	ata	aat	ata	gca	gga	caa	aca	act	gta	aac	gca	aac	ttt	caa	aca	2304
5	Lys	Ile	Asn	Ile	Ala	Gly	Gln	Thr	Thr	Val	Asn	Ala	Asn	Phe	Gln	Thr	
			755					760					765				
	aat	ctt	ttt	gaa	aaa	aat	att	aat	ata	act	act	ctt	aat	tta	aaa	aat	2352
10	Asn	Leu	Phe	Glu	Lys	Asn	Ile	Asn	Ile	Thr	Thr	Leu	Asn	Leu	Lys	Asn	
		770					775					780					
	att	caa	aaa	aat	ata	gga	att	aag	ctc	ctt	cag	cca	ata	aaa	att	ata	2400
	Ile	Gln	Lys	Asn	Ile	Gly	Ile	Lys	Leu	Leu	Gln	Pro	Ile	Lys	Ile	Ile	
15		785				790				795				800			
	gtc	tca	cct	caa	caa	ttt	gtt	ctt	aat	aac	tgt	tca	cta	gca	att	ctt	2448
	Val	Ser	Pro	Gln	Gln	Phe	Val	Leu	Asn	Asn	Cys	Ser	Leu	Ala	Ile	Leu	
				805					810				815				
	cca	tct	gga	aca	att	aca	act	gat	ata	tat	gtt	act	cct	caa	cga	ctt	2496
20	Pro	Ser	Gly	Thr	Ile	Thr	Thr	Asp	Ile	Tyr	Val	Thr	Pro	Gln	Arg	Leu	
			820					825					830				
	aat	gct	aat	gca	atc	att	aaa	gaa	gtt	tca	ctt	ctc	tct	ttc	caa	cca	2544
25	Asn	Ala	Asn	Ala	Ile	Ile	Lys	Glu	Val	Ser	Leu	Leu	Ser	Phe	Gln	Pro	
		835					840					845					
	ttt	agt	ata	ctt	ctt	cct	caa	gga	aat	ata	aat	gga	cac	ata	aca	ctt	2592
30	Phe	Ser	Ile	Leu	Leu	Pro	Gln	Gly	Asn	Ile	Asn	Gly	His	Ile	Thr	Leu	
		850				855					860						
	aca	gga	ata	cct	agt	aaa	cct	aaa	gga	aca	ctc	tca	ttt	gat	att	cta	2640
	Thr	Gly	Ile	Pro	Ser	Lys	Pro	Lys	Gly	Thr	Leu	Ser	Phe	Asp	Ile	Leu	
35		865				870				875			880				
	aac	ata	cat	tat	cca	agg	cca	aat	cca	tca	ata	gca	aac	tta	cat	gta	2688
	Asn	Ile	His	Tyr	Pro	Arg	Pro	Asn	Pro	Ser	Ile	Ala	Asn	Leu	His	Val	
			885				890					895					
	gaa	ggg	gaa	att	ata	tct	tct	cct	aac	aat	ata	tgt	aaa	ctt	aat	gca	2736
40	Glu	Gly	Glu	Ile	Ile	Ser	Ser	Pro	Asn	Asn	Ile	Cys	Lys	Leu	Asn	Ala	
			900					905				910					
	acc	cta	aca	gaa	aaa	aaa	gag	cct	ata	cct	ata	tca	ata	caa	gca	aca	2784
45	Thr	Leu	Thr	Glu	Lys	Lys	Glu	Pro	Ile	Pro	Ile	Ser	Ile	Gln	Ala	Thr	



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	915	920	925	
	ctc cct ttt gag ttc aca gaa aac aat atc cct atg cta tct aaa atg			2832
	Leu Pro Phe Glu Phe Thr Glu Asn Asn Ile Pro Met Leu Ser Lys Met			
5	930	935	940	
	agg cct ttt tct gcc cat atc aag tgg act gga ata tta gat aca ctt			2880
	Arg Pro Phe Ser Ala His Ile Lys Trp Thr Gly Ile Leu Asp Thr Leu			
	945	950	955	960
10				
	tgg aaa ctc att cca ctt act gat tac att atg gct ggg aat gga tct			2928
	Trp Lys Leu Ile Pro Leu Thr Asp Tyr Ile Met Ala Gly Asn Gly Ser			
		965	970	975
15				
	tta gat gct tct ctt tct ggg act tta gat agt cca aca tat gca att			2976
	Leu Asp Ala Ser Leu Ser Gly Thr Leu Asp Ser Pro Thr Tyr Ala Ile			
		980	985	990
20				
	ata aca aca ctt tct aat gct aac ttt caa gat ctc tcc ctt ggt ctt			3024
	Ile Thr Thr Leu Ser Asn Ala Asn Phe Gln Asp Leu Ser Leu Gly Leu			
		995	1000	1005
25				
	tac tta gaa aat atc aat gct aaa tta cag gtc ttt tct aat aga atc			3072
	Tyr Leu Glu Asn Ile Asn Ala Lys Leu Gln Val Phe Ser Asn Arg Ile			
	1010	1015	1020	
30				
	tcc cat att caa gct aca gca tct gat ggt aaa caa ggt agt ata caa			3120
	Ser His Ile Gln Ala Thr Ala Ser Asp Gly Lys Gln Gly Ser Ile Gln			
	1025	1030	1035	1040
	ctt att ggt aat att ggc tca tct aaa gaa cac ttt cct ttg tct att			3168
	Leu Ile Gly Asn Ile Gly Ser Ser Lys Glu His Phe Pro Leu Ser Ile			
		1045	1050	1055
35				
	aat ggc tcc ttt aca aac ctt gct cca tta caa cgt aaa gac cta agt			3216
	Asn Gly Ser Phe Thr Asn Leu Ala Pro Leu Gln Arg Lys Asp Leu Ser			
		1060	1065	1070
40				
	ctt aca ctt tca gga gca gct act ctt gaa gga aca tta aaa cag tct			3264
	Leu Thr Leu Ser Gly Ala Ala Thr Leu Glu Gly Thr Leu Lys Gln Ser			
		1075	1080	1085
45				
	gaa gtt aaa ggc gat att gtt att aac caa ggc gaa ttt caa ctt act			3312
	Glu Val Lys Gly Asp Ile Val Ile Asn Gln Gly Glu Phe Gln Leu Thr			
	1090	1095	1100	

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5      gaa ggg tta acc agt aat att cca act ctt aat gta gtt gat agc act      3360  
       Glu Gly Leu Thr Ser Asn Ile Pro Thr Leu Asn Val Val Asp Ser Thr  
       1105                      1110                      1115                      1120

10      caa caa caa aat aca aag acc aaa aaa gct acc tat caa caa cct acc      3408  
       Gln Gln Gln Asn Thr Lys Thr Lys Lys Ala Thr Tyr Gln Gln Pro Thr  
                                  1125                      1130                      1135

10      tta tct att gcg tta agt atc ccg aat cgt ttt ttt gtc cgt agt agt      3456  
       Leu Ser Ile Ala Leu Ser Ile Pro Asn Arg Phe Phe Val Arg Ser Ser  
                                  1140                      1145                      1150

15      atg ttt gaa agt gag tgg gga ggg aac cta act att aac aaa gtc ata      3504  
       Met Phe Glu Ser Glu Trp Gly Gly Asn Leu Thr Ile Asn Lys Val Ile  
                                  1155                      1160                      1165

20      aca agt cct gtt att aca gga gca cta act tct ata aga gga aat ttt      3552  
       Thr Ser Pro Val Ile Thr Gly Ala Leu Thr Ser Ile Arg Gly Asn Phe  
                                  1170                      1175                      1180

25      aat tta cta gga aaa caa ttt tct ctt gct aaa agt aca ata tca ttt      3600  
       Asn Leu Leu Gly Lys Gln Phe Ser Leu Ala Lys Ser Thr Ile Ser Phe  
                                  1185                      1190                      1195                      1200

30      tca gga tca gtt cca cca aac cca cta ctc aat att tct tta aca tat      3648  
       Ser Gly Ser Val Pro Pro Asn Pro Leu Leu Asn Ile Ser Leu Thr Tyr  
                                  1205                      1210                      1215

30      tca tca cct tct att aca gct ata ggc att att aaa ggt aca act agt      3696  
       Ser Ser Pro Ser Ile Thr Ala Ile Gly Ile Ile Lys Gly Thr Thr Ser  
                                  1220                      1225                      1230

35      aat cct aat att act ttt tca agt aca cca cct tta cct caa gat gaa      3744  
       Asn Pro Asn Ile Thr Phe Ser Ser Thr Pro Pro Leu Pro Gln Asp Glu  
                                  1235                      1240                      1245

40      ata gtt tcc caa gtt ctt ttt ggt aaa agc tca caa agt ctt agc agg      3792  
       Ile Val Ser Gln Val Leu Phe Gly Lys Ser Ser Gln Ser Leu Ser Arg  
                                  1250                      1255                      1260

45      ata caa gcc ata caa ctt gct caa gaa tta gca aac tta aca gga ttt      3840  
       Ile Gln Ala Ile Gln Leu Ala Gln Glu Leu Ala Asn Leu Thr Gly Phe  
                                  1265                      1270                      1275                      1280

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	aat act gga agt atg aat ttc cta aca aat att cga cag aca tta ggg	3888
	Asn Thr Gly Ser Met Asn Phe Leu Thr Asn Ile Arg Gln Thr Leu Gly	
	1285 1290 1295	
5	tta gat ata ctt agc tta ggg aca act tct aat aga aaa gcc aat aca	3936
	Leu Asp Ile Leu Ser Leu Gly Thr Thr Ser Asn Arg Lys Ala Asn Thr	
	1300 1305 1310	
10	tcc aac tca aac gat caa ata gaa gat atc cct gtt ata gaa cta ggt	3984
	Ser Asn Ser Asn Asp Gln Ile Glu Asp Ile Pro Val Ile Glu Leu Gly	
	1315 1320 1325	
15	aaa tat att aca gac act gtt tat gtt ggt gtt gaa caa agt tat tta	4032
	Lys Tyr Ile Thr Asp Thr Val Tyr Val Gly Val Glu Gln Ser Tyr Leu	
	1330 1335 1340	
20	gat agt aat gat act ggg gca aga ata tca gtt gaa ctt gca cct aat	4080
	Asp Ser Asn Asp Thr Gly Ala Arg Ile Ser Val Glu Leu Ala Pro Asn	
	1345 1350 1355 1360	
	ttt aat ctt gaa ggt aga aca ggg act caa tat agt gag ata ggt att	4128
	Phe Asn Leu Glu Gly Arg Thr Gly Thr Gln Tyr Ser Glu Ile Gly Ile	
	1365 1370 1375	
25	aat tgg aaa aaa gat tat taa	4149
	Asn Trp Lys Lys Asp Tyr	
	1380	
30	<210> 18	
	<211> 1382	
	<212> PRT	
	<213> Lawsonia intracellularis	
35	<400> 18	
	Met Asn Asn Thr Lys Ile Leu Ser Lys Leu Leu Tyr Thr Leu Leu Gly	
	1 5 10 15	
40	Ala Phe Thr Leu Phe Leu Gly Leu Ile Ile Thr Gly Ile Leu Phe Ile	
	20 25 30	
	Arg Thr Ser Thr Gly Ile Ala Trp Ile Lys Asn Thr Val Ser Ser Leu	
	35 40 45	
45	Leu Gln Gln Gln Gly Ile Ile Leu Gln Val Ser Ser Ile Ile Gly Pro	

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	50		55		60
	Phe Pro Glu Gln Ile Thr Ile Asn Glu Leu Ser Leu Ser Asp Val Asn				
	65		70		75 80
5	Gly Thr Tyr Leu Thr Ile Ser Asn Leu Glu Ile Gln Ser Asn Leu Trp				
		85		90	95
	Ala Leu Phe Lys Gly Gln Leu Glu Ile Leu Ser Phe Glu Leu Asn Asp				
10		100		105	110
	Leu Val Leu Tyr Arg Leu Pro Ser Asn Asn Asn Leu Lys Lys Ser Ser				
		115		120	125
15	Thr Ser Phe Val Leu Pro His Ile Ser Phe Asp Leu Thr Pro Trp Trp				
		130		135	140
	Thr Glu His Ile Arg Ile Gln Asn Ile His Ile Asn Asn Thr Gln Leu				
	145		150		155 160
20	Ser Ser Asp Ile Ile Gly Ile Pro Leu Val Leu Ser Leu Glu Gly Asp				
		165		170	175
	Gly Thr Leu Thr Asn Trp Asn Gly Thr Phe Gln Leu Ser Ser Ser Asn				
25		180		185	190
	Lys Thr Lys Ile Ile Gly Thr Leu Arg Tyr Gln Gly Asn Lys Thr Gln				
		195		200	205
30	Phe Phe Glu Tyr Val His Pro Thr Arg Ile Val Thr Leu Glu Ile Asp				
		210		215	220
	Ser Val Ala Asp Lys Lys Ser Tyr Asn Asn Ser Ile Leu Glu Gln Pro				
	225		230		235 240
35	Leu His Leu His Leu Ser Ile Tyr Pro Glu His Asn Arg Ile Ile Leu				
		245		250	255
	His Ser Leu Leu Ala Glu Tyr Gly Ser Trp Leu Leu Thr Ser Glu Ser				
40		260		265	270
	Ile Glu Val Ser Asn Glu Gln Leu Lys Gly Asn Ile Leu Leu Lys Tyr				
		275		280	285
45	Asn Gly Glu Ala Thr His Gln Leu Pro Ile Lys Lys Leu Asn Ser Ser				

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	290	295	300
	Ile Thr Leu Ser Gly Ser Leu Asn Lys Pro Asn Phe Ser Ile Gln Met		
	305	310	315 320
5	Thr Leu Pro Glu Ile Asn Ile Thr Lys Asn Ile Ile Asp Leu Gln Thr		
	325	330	335
10	Glu Leu Val Ile Asn Leu Gly Leu Phe Ser Thr His Ser Asp Ile Leu		
	340	345	350
	Thr Ser Gly Thr Ile Thr Val Gln Gly Glu Thr Ile Pro Asn Ser Ile		
	355	360	365
15	Leu Ser Ser Ala Val Asp Ile Ile Ala Ser Thr Thr Thr His Thr Ile		
	370	375	380
	Thr Leu Glu His Ala Thr Leu Thr Ser Pro Glu Met His Phe Ser Leu		
	385	390	395 400
20	Ser Gly Glu Phe Asn Ser Leu Leu Gly Asn Ile Asp Ala Asn Leu Lys		
	405	410	415
	Gly Asn Thr Pro Thr Leu Ser Ile Phe Ser Ser Leu Leu Gly Leu Pro		
25	420	425	430
	Asp Leu Thr Gly Gln Ser Asn Ile Thr Ile Gly Leu His Arg Gln Gly		
	435	440	445
30	Ser Ser Ser Ser Ile Glu Gly Thr Ala Thr Val Ser Leu Asn Asn Met		
	450	455	460
	Asn Trp Gly Val Gln Ala Leu Gln Gly Thr Leu Gly Asp Asn Ala Thr		
	465	470	475 480
35	Leu Ser Gly Ile Tyr Asn Leu Thr Pro Ile Asp Trp Ser Ile Ser Leu		
	485	490	495
	Asn Lys Leu Lys Leu Thr Ala Lys Asn Val Tyr Ala Glu Gly Leu Ile		
40	500	505	510
	Asn Phe Gln Lys Lys Tyr Ile Asp Ser Ser Ile Asn Leu Ile Ile Pro		
	515	520	525
45	Asn Leu Gln Leu Ile Ala Pro Pro Ile Ser Gly Glu Leu Gln Ser Leu		

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	530	535	540
	Ile Thr Val Ser Gly Lys Leu Asp Ala Pro Ser Ile Glu Ser Lys Ile		
	545	550	555 560
5	Phe Ser Ser Gln Leu Thr Trp Asn Ala Leu Gln Leu Asn Asn Pro Gln		
	565	570	575
	Leu Ile Ile Thr Thr Thr Gln Ser Ser Ser Ser Ala Ile Lys Gly Asn		
10	580	585	590
	Ile Thr Leu Ser Ala Glu Pro Ala Ser Ser Glu Ala Leu Thr Phe Ser		
	595	600	605
15	Ser Asn Trp Gly Ile Leu Pro Thr Glu Ile Leu Val Glu Lys Ile Ile		
	610	615	620
	Gly Asn Ile Leu Gly Val Asn Leu Asp Gly Asn Ile Lys Ile Thr Lys		
20	625	630	635 640
	Lys Asp Tyr Leu Ile Asn Gly Asp Ile Ile Ala Glu Val Gln Ser Trp		
	645	650	655
	Lys Asp Ile Ala Asn Ile Leu Gln Ile Pro Ile Arg Gly Ser Ala Ser		
25	660	665	670
	Ile Lys Ile Gln Phe Asp Pro Lys Asn Gln Gln Cys Ile Ser Thr Gln		
	675	680	685
30	Trp Gln Leu Lys Asn Phe Ile Leu Gly Asn Asn Phe Asn Val Thr Thr		
	690	695	700
	Ile Lys Gly Arg Ala Asp Thr Ile Gln Leu His Lys Asn Pro Thr Ile		
35	705	710	715 720
	Ala Leu Ser Ser Lys Ile Gly Ala Gly Thr Tyr Glu Asp Phe Gln Trp		
	725	730	735
	Thr Gln Gly Thr Leu Asp Ile Lys Gly Thr Leu Lys Asn Phe Asn Ser		
40	740	745	750
	Lys Ile Asn Ile Ala Gly Gln Thr Thr Val Asn Ala Asn Phe Gln Thr		
	755	760	765
45	Asn Leu Phe Glu Lys Asn Ile Asn Ile Thr Thr Leu Asn Leu Lys Asn		

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	770	775	780
	Ile Gln Lys Asn Ile Gly Ile Lys Leu Leu Gln Pro Ile Lys Ile Ile		
	785	790	795 800
5	Val Ser Pro Gln Gln Phe Val Leu Asn Asn Cys Ser Leu Ala Ile Leu		
	805	810	815
	Pro Ser Gly Thr Ile Thr Thr Asp Ile Tyr Val Thr Pro Gln Arg Leu		
10	820	825	830
	Asn Ala Asn Ala Ile Ile Lys Glu Val Ser Leu Leu Ser Phe Gln Pro		
	835	840	845
15	Phe Ser Ile Leu Leu Pro Gln Gly Asn Ile Asn Gly His Ile Thr Leu		
	850	855	860
	Thr Gly Ile Pro Ser Lys Pro Lys Gly Thr Leu Ser Phe Asp Ile Leu		
	865	870	875 880
20	Asn Ile His Tyr Pro Arg Pro Asn Pro Ser Ile Ala Asn Leu His Val		
	885	890	895
	Glu Gly Glu Ile Ile Ser Ser Pro Asn Asn Ile Cys Lys Leu Asn Ala		
25	900	905	910
	Thr Leu Thr Glu Lys Lys Glu Pro Ile Pro Ile Ser Ile Gln Ala Thr		
	915	920	925
30	Leu Pro Phe Glu Phe Thr Glu Asn Asn Ile Pro Met Leu Ser Lys Met		
	930	935	940
	Arg Pro Phe Ser Ala His Ile Lys Trp Thr Gly Ile Leu Asp Thr Leu		
	945	950	955 960
35	Trp Lys Leu Ile Pro Leu Thr Asp Tyr Ile Met Ala Gly Asn Gly Ser		
	965	970	975
	Leu Asp Ala Ser Leu Ser Gly Thr Leu Asp Ser Pro Thr Tyr Ala Ile		
40	980	985	990
	Ile Thr Thr Leu Ser Asn Ala Asn Phe Gln Asp Leu Ser Leu Gly Leu		
	995	1000	1005
45	Tyr Leu Glu Asn Ile Asn Ala Lys Leu Gln Val Phe Ser Asn Arg Ile		

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	1010	1015	1020
	Ser His Ile Gln Ala Thr Ala Ser Asp Gly Lys Gln Gly Ser Ile Gln		
	1025	1030	1035 1040
5	Leu Ile Gly Asn Ile Gly Ser Ser Lys Glu His Phe Pro Leu Ser Ile		
	1045	1050	1055
	Asn Gly Ser Phe Thr Asn Leu Ala Pro Leu Gln Arg Lys Asp Leu Ser		
10	1060	1065	1070
	Leu Thr Leu Ser Gly Ala Ala Thr Leu Glu Gly Thr Leu Lys Gln Ser		
	1075	1080	1085
15	Glu Val Lys Gly Asp Ile Val Ile Asn Gln Gly Glu Phe Gln Leu Thr		
	1090	1095	1100
	Glu Gly Leu Thr Ser Asn Ile Pro Thr Leu Asn Val Val Asp Ser Thr		
20	1105	1110	1115 1120
	Gln Gln Gln Asn Thr Lys Thr Lys Lys Ala Thr Tyr Gln Gln Pro Thr		
	1125	1130	1135
	Leu Ser Ile Ala Leu Ser Ile Pro Asn Arg Phe Phe Val Arg Ser Ser		
25	1140	1145	1150
	Met Phe Glu Ser Glu Trp Gly Gly Asn Leu Thr Ile Asn Lys Val Ile		
	1155	1160	1165
30	Thr Ser Pro Val Ile Thr Gly Ala Leu Thr Ser Ile Arg Gly Asn Phe		
	1170	1175	1180
	Asn Leu Leu Gly Lys Gln Phe Ser Leu Ala Lys Ser Thr Ile Ser Phe		
35	1185	1190	1195 1200
	Ser Gly Ser Val Pro Pro Asn Pro Leu Leu Asn Ile Ser Leu Thr Tyr		
	1205	1210	1215
	Ser Ser Pro Ser Ile Thr Ala Ile Gly Ile Ile Lys Gly Thr Thr Ser		
40	1220	1225	1230
	Asn Pro Asn Ile Thr Phe Ser Ser Thr Pro Pro Leu Pro Gln Asp Glu		
	1235	1240	1245
45	Ile Val Ser Gln Val Leu Phe Gly Lys Ser Ser Gln Ser Leu Ser Arg		

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1250

1255

1260

Ile Gln Ala Ile Gln Leu Ala Gln Glu Leu Ala Asn Leu Thr Gly Phe  
 1265 1270 1275 1280

5

Asn Thr Gly Ser Met Asn Phe Leu Thr Asn Ile Arg Gln Thr Leu Gly  
 1285 1290 1295

Leu Asp Ile Leu Ser Leu Gly Thr Thr Ser Asn Arg Lys Ala Asn Thr  
 1300 1305 1310

10

Ser Asn Ser Asn Asp Gln Ile Glu Asp Ile Pro Val Ile Glu Leu Gly  
 1315 1320 1325

15

Lys Tyr Ile Thr Asp Thr Val Tyr Val Gly Val Glu Gln Ser Tyr Leu  
 1330 1335 1340

Asp Ser Asn Asp Thr Gly Ala Arg Ile Ser Val Glu Leu Ala Pro Asn  
 1345 1350 1355 1360

20

Phe Asn Leu Glu Gly Arg Thr Gly Thr Gln Tyr Ser Glu Ile Gly Ile  
 1365 1370 1375

Asn Trp Lys Lys Asp Tyr  
 1380

25

&lt;210&gt; 19

&lt;211&gt; 26

&lt;212&gt; DNA

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&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:

Oligonucleotide probe/primer

35

&lt;400&gt; 19

catattcaag gtacagcatc tgatgg

26

&lt;210&gt; 20

40

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

45

&lt;223&gt; Description of Artificial Sequence:

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Oligonucleotide probe/primer

	<400> 20	
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5		
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	<220>	
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	<400> 21	
	gctcatctaa agaacacttt cc	22
20	<210> 22	
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	<400> 22	
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	<210> 23	
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	<220>	
	<223> Description of Artificial Sequence:	
40	Oligonucleotide probe/primer	
	<400> 23	
	gacctaagtc ttacactttc agg	23
45		

•

45 &lt;220&gt;

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<223> Description of Artificial Sequence:  
Oligonucleotide probe/primer

<400> 27  
5 aagaatgcct gtaataataa gtcc 24

<210> 28  
<211> 19  
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15 Oligonucleotide probe/primer

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30 <400> 29  
tattaggagt aaatcttgat g 21

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Oligonucleotide probe/primer

45  
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<210> 31  
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<220>  
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<211> 24  
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<220>  
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Oligonucleotide probe/primer

45 <400> 44



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<210> 45

5 <211> 21

<212> DNA

<213> Artificial Sequence

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10 <223> Description of Artificial Sequence:  
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15

<210> 46

<211> 23

<212> DNA

20 <213> Artificial Sequence

<220>

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25

<400> 46

caactccact tcaagataga agc

23



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**WE CLAIM:**

1. An isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of a *Lawsonia spp.* polypeptide selected from the group consisting of flhB, fliR, ntrC, glhH, motA, motB, tlyC, ytfM, and ytfN polypeptides.
2. The isolated or recombinant immunogenic polypeptide of claim 1 capable of eliciting the production of antibodies against *Lawsonia spp.* when administered to an avian or porcine animal.
3. The isolated or recombinant immunogenic polypeptide of claim 1 capable of conferring a protective immune response against *Lawsonia spp.* when administered to an avian or porcine animal.
4. The isolated or recombinant immunogenic polypeptide of claim 2 wherein the *Lawsonia spp.* is *L. intracellularis*.
5. The isolated or recombinant immunogenic polypeptide of claim 3 wherein the *Lawsonia spp.* is *L. intracellularis*.
6. An isolated or recombinant polypeptide selected from the group consisting of:
  - (i) a polypeptide of *Lawsonia spp.* which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;
  - (ii) a polypeptide of *Lawsonia spp.* which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence encoded by *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glhH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480

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(plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(iv) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence encoded by *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glhH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(v) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence of *Lawsonia spp.* having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(vi) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence of *Lawsonia spp.* having at least about 60% sequence identity overall to the nucleotide sequence of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glhH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(vii) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(viii) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glhH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478



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(plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(viii) a homologue, analogue or derivative of any one of (i) to (vii) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

7. The isolated or recombinant polypeptide of claim 6 capable of eliciting the production of antibodies against *Lawsonia spp.* in a porcine or avian animal.
8. The isolated or recombinant polypeptide of claim 7 capable of conferring a protective immune response against *Lawsonia spp.* in a porcine or avian animal.
9. The isolated or recombinant polypeptide of claim 8, capable of inducing humoral immunity against *Lawsonia spp.* in a porcine or avian animal.
10. The isolated or recombinant polypeptide of claim 9, capable of inducing humoral immunity against *Lawsonia spp.* in a porcine animal.
11. The isolated or recombinant polypeptide of claim 8 wherein the *Lawsonia spp.* is *Lawsonia intracellularis*.
12. The isolated or recombinant polypeptide of claim 10 wherein the *Lawsonia spp.* is *Lawsonia intracellularis*.
13. The isolated or recombinant polypeptide of claim 6 comprising an amino acid sequence selected from the group consisting of:
  - (i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18; and
  - (ii) an amino acid sequence encoded by *Lawsonia intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid

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pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

14. The isolated or recombinant polypeptide of claim 13 capable of eliciting the production of antibodies against *Lawsonia intracellularis* when administered to an avian or porcine animal.

15. The isolated or recombinant polypeptide of claim 13 or 14 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine or avian animal.

16. The isolated or recombinant polypeptide of claim 15 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine animal.

17. A vaccine composition for the prophylaxis or treatment of infection of an animal by *Lawsonia spp.*, said vaccine composition comprising an immunogenic component which comprises the isolated or recombinant immunogenic polypeptide according to claim 1 in combination with one or more carriers, diluents or adjuvants suitable for veterinary or pharmaceutical use.

18. The vaccine composition according to claim 17 wherein the *Lawsonia spp.* is *Lawsonia intracellularis*.

19. The vaccine composition according to claim 17 wherein the immunogenic component comprises an isolated or recombinant polypeptide having an amino acid sequence selected from the group consisting of:

- (i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18; and
- (ii) an amino acid sequence encoded by *Lawsonia intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL



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Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

20. The vaccine composition of claim 17, wherein the immunogenic component is a recombinant polypeptide expressed in a cell that has been transfected with a vector comprising a nucleotide sequence selected from the group consisting of:

- (i) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;
- (ii) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to the protein-encoding sequence of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);
- (iii) a protein-encoding nucleotide sequence which comprises at least about 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;
- (iv) a protein-encoding nucleotide sequence which comprises at least about 15 contiguous nucleotides of the protein-encoding sequence of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);
- (v) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the complement of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

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- (vi) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the non-coding strand of *Lawsonia intracellularis* DNA contained within a p plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and
- (vii) a homologue, analogue or derivative of any one of (i) to (vi) which encodes a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

21. A combination vaccine composition for the prophylaxis or treatment of infection of an animal by *Lawsonia spp.*, said vaccine composition comprising:

- (i) a first immunogenic component which comprises the isolated or recombinant polypeptide having according to claim 1;
- (ii) a second immunogenic component different from said first immunogenic component and comprising a polypeptide selected from the group consisting of the *Lawsonia intracellularis* FlgE, hemolysin, OmpH, SodC, flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides; and
- (iii) one or more carriers, diluents or adjuvants suitable for veterinary or pharmaceutical use.

22. A vaccine vector that comprises, in an expressible form, an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- (i) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;
- (ii) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to the protein-encoding sequence of *Lawsonia intracellularis* DNA contained within a p plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid



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pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a protein-encoding nucleotide sequence which comprises at least about 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a protein-encoding nucleotide sequence which comprises at least about 15 contiguous nucleotides of the protein-encoding sequence of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the complement of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(vi) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the non-coding strand of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(vii) a homologue, analogue or derivative of any one of (i) to (vi) which encodes a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

wherein said vaccine vector expresses the polypeptide encoded by said nucleotide sequence at a level sufficient to confer immunity against *Lawsonia spp.* when administered to a porcine or avian animal.

23. The vaccine vector of claim 22 wherein the immunogenic polypeptide is expressed by a process comprising:

(i) placing an isolated nucleic acid molecule in an expressible format, said

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nucleic acid molecule comprising the coding region of a gene selected from the group consisting of *flhB*, *fliR*, *ntnC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes, or a protein-encoding homologue, analogue or derivative thereof;

(ii) introducing the isolated nucleic acid molecule of (i) in an expressible format into a suitable vaccine vector; and

(iii) incubating or growing the vaccine vector for a time and under conditions sufficient for expression of the immunogenic component encoded by said nucleic acid molecule to occur.

24. The vaccine vector of claim 22 wherein the *Lawsonia spp.* is *L. intracellularis*.

25. An isolated polyclonal antibody or a monoclonal antibody molecule that binds specifically to a *Lawsonia spp.* polypeptide selected from the group consisting of *flhB*, *fliR*, *ntnC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* polypeptides, or a homologue, analogue or derivative of any one or more of said polypeptides.

26. The isolated polyclonal antibody or a monoclonal antibody molecule of claim 25 wherein the polypeptide or derivative thereof comprises an amino acid sequence selected from the group consisting of:

(i) an amino acid sequence of *Lawsonia sp.* which has at least about 60% sequence identity overall to a sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(ii) an amino acid sequence of *Lawsonia sp.* which has at least about 60% sequence identity overall to a sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 *glnH*); NM00/16477 (plasmid pGTE#2 *flhB*); NM00/16478 (plasmid pGTE#3 *fliR*); NM00/16479 (plasmid pGTE#4 *motA/B*); NM00/16480 (plasmid pGTE#5 *tlyC*); NM00/16481 (plasmid pGTE#6 *ntnC*); and NM00/16482 (plasmid pGTE#7 *ytfM*);

(iii) an amino acid sequence which comprises at least about 5 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6,

8, 10, 12, 14, 16, and 18;

(iv) an amino acid sequence which comprises at least about 5 contiguous amino acids of a sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) an amino acid sequence which is encoded by a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(vi) an amino acid sequence which is encoded by a nucleotide sequence having at least about 60% sequence identity overall to the nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(vii) an amino acid sequence which is encoded by at least about 15 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(viii) an amino acid sequence which is encoded by at least about 15 contiguous nucleotides of a nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(ix) a homologue, analogue or derivative of any one of (i) to (viii) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

27. A method of diagnosing infection of a porcine or avian animal by *Lawsonia intracellularis* or a microorganism that is immunologically cross-reactive thereto, said method comprising the steps of contacting a biological sample derived from said animal with the antibody molecule of claim 25 for a time and under conditions sufficient for an antigen:antibody complex to form, and then detecting said complex formation.

28. The method of claim 27 wherein the biological sample comprises whole serum, lymph nodes, ileum, caecum, small intestine, large intestine, faeces or a rectal swab derived from a porcine animal.

29. A method of identifying whether or not a porcine or avian animal has suffered from a past infection, or is currently infected, with *Lawsonia intracellularis* or a microorganism that is immunologically cross-reactive thereto, said method comprising contacting blood or serum derived from said animal with the immunogenic polypeptide of claim 1 for a time and under conditions sufficient for an antigen:antibody complex to form and then detecting said complex formation.

30. An isolated nucleic acid molecule which consists of a nucleotide sequence encoding a *Lawsonia spp.* polypeptide selected from the group consisting of flhB, flhR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN.

31. The isolated nucleic acid molecule according to claim 30 comprising a sequence of nucleotides selected from the group consisting of:

- (i) a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;
- (ii) a nucleotide sequence having at least about 60% sequence identity overall to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR);

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NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);  
 (iii) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);  
 (v) a nucleotide sequence which hybridizes under at least low stringency conditions to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17 or a complementary nucleotide sequence thereto;

(vi) a nucleotide sequence which hybridizes under at least low stringency conditions to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 flhR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);  
 and

(vii) a homologue, analogue or derivative of any one of (i) to (vi) which encodes a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

32. The isolated nucleic acid molecule of claim 31 comprising a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;
- (ii) a nucleotide sequence of the *L. intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL Accession Nos:

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NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(iii) a nucleotide sequence that encodes the same polypeptide as a nucleotide sequence of (i) or (ii), wherein said polypeptide is selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides; and

(iv) a nucleotide sequence that is complementary to a nucleotide sequence of (i) or (ii) or (iii).

33. The isolated nucleic acid molecule of claim 32 consisting of the protein-encoding region of (i) or (ii).

34. A method of detecting *Lawsonia intracellularis* or related microorganism in a biological sample derived from a porcine or avian animal subject, said method comprising the steps of hybridising one or more probes or primers to said sample and then detecting said hybridisation using a detection means, wherein said probes or primers are derived from a *Lawsonia spp.* gene selected from the group consisting of *flhB*, *fliR*, *ntrC*, *glnH*, *motA*, *motB*, *tlyC*, *ytfM*, and *ytfN* genes.

35. The method of claim 34 wherein the biological sample comprises whole serum, lymph nodes, ileum, caecum, small intestine, large intestine, faeces or a rectal swab derived from a porcine animal.

36. The method of claim 34 wherein the detection means comprises any nucleic acid based hybridisation or amplification reaction.

37. A probe or primer comprising a nucleotide sequence selected from the group consisting of:

(i) any one of SEQ ID NOs: 19 to 46; and

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(ii) a complementary nucleotide sequence to (i).

38. A plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).
39. A recombinant vector capable of replication in a host cell, wherein said vector comprises the isolated nucleic acid of claim 30.
40. A recombinant vector capable of replication in a host cell, wherein said vector comprises the isolated nucleic acid of claim 31.
41. A recombinant vector capable of replication in a host cell, wherein said vector comprises the isolated nucleic acid of claim 32.
42. A recombinant vector capable of replication in a host cell, wherein said vector comprises the isolated nucleic acid of claim 33.
43. A host cell comprising the recombinant vector of claim 39.
44. The host cell of claim 43 wherein said host cell is a bacterium.
45. A host cell comprising the recombinant vector of claim 40.
46. The host cell of claim 45 wherein said host cell is a bacterium.
47. A host cell comprising the recombinant vector of claim 41.
48. The host cell of claim 47 wherein said host cell is a bacterium.

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49. A host cell comprising the recombinant vector of claim 42.

50. The host cell of claim 49 wherein said host cell is a bacterium.

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*Australian Pork Limited*  
**Agriculture Victoria Services Pty. Ltd. AND ~~Pig Research and Development~~  
Corporation AND Pfizer Products, Inc.**

by **DAVIES COLLISON CAVE**  
Patent Attorneys for the applicant(s)



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